# Erythropoietin Level and Hematologic Parameters in Healthy Adults†

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**Abstract**

Since the major physiological control of erythropoiesis is related to the eryhtropoietin (EPO) level, correlating the EPO level with hematologic parameters in healthy adults, which constitutes an inexpensive and simple routine laboratory report, would be very useful. Two hundred healthy adult blood donors, 100 males and 100 females, aged between 17 and 60 years old were randomly chosen. The EPO reference range was determined by enzyme linked immmunosorbent assay (ELISA) using a reagent kit from Research & Development Systems Inc. The hematologic values for reticulocyte and red blood cell parameters were assessed using the Technicon H*3 RTC, an automated blood cell analyzer. The EPO reference range in the studied population was 2.21-20.95 mU/ml. The correlations between the EPO level and hematologic parameters were between -0.302 ($p<0.01$) and 0.294 ($p<0.01$). The results suggested that there were none or low correlations between the EPO level and hematologic parameters in healthy adults. According to our results, these parameters could not be used to indicate the level of EPO in healthy adults.

**Key word :** Erythropoietin, Reference Range, Correlation Study, Hematologic Parameters, Healthy Adults

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Erythropoietin (EPO) is a 30,400 MW glycoprotein produced mainly by the peritubular cells of the kidney and has a predominant effect on the committed erythroid cells, colony-forming-unit erythroids, promoting their proliferation and differentiation into proerythroblasts[1]. Therefore, determining the level of EPO is a reflection of bone marrow erythropoietic activity. Although the reference level of EPO is the important baseline parameter to understand the physiology of erythropoiesis, there is no reference level of EPO in Thai adults. There were reports[2,3] suggesting that the physiological stimulus for EPO production was tissue hypoxia, which in most instance was directly related to the number of circulating eryhtrocytes. Nowadays, with the development of a sensitive and accurate assay, EPO can be detected in the serum. However, the assay technique is expensive and not practical. Recent studies[4-6] demonstrated the capability of hematologic parameters generated from an automated blood cell analyzer, which was widely available in the routine hematology laboratory, that could be used to indicate the response to recombinant human EPO (rHu-EPO) in anemic patients. The correlation between the EPO level and hematologic parameters still constitutes one of the open questions in healthy persons.

The purpose of this study was to determine the reference range of EPO level in Thai adults. In addition, the correlation between the serum EPO levels and routine red blood cell parameters would be analyzed with the aim of using routine red blood cell parameters, which are cheaper and more convenient to determine, to indicate the serum EPO levels in this population.

## MATERIAL AND METHOD

### Samples and Subjects:

Two hundred subjects (100 males and 100 females) were recruited from blood donors of the Thai Red Cross between January and May 1997. Age, gender and history of illness of each subject were noted at the time of the sample collection. The inclusion criteria were those subjects without any history of illness, which was likely to influence the complete blood count (CBC). All subjects had normal results in terms of blood pressure and blood examinations according to the Thai Red Cross criteria for blood donors. No anemia was found using the WHO criteria[7]. The exclusion criteria were those subjects who had abnormal blood pressure or blood tests, such as anemia or were flagged on CBC reported by Technicon H*3 RTC. Since the state of erythropoiesis can be assumed to have been in a steady state within 3 months after the last blood donation, the subjects who had donated blood within 4 months prior to the study were also excluded in order to avoid any interference resulting from the recent donation.

Blood samples of healthy adults were drawn into clotted blood tubes and tubes coated with tripotassium ethylene diamine tetraacetic acid ($K_3$ EDTA) before donation. Using evacuated tubes (Venoject), 3 ml of clotted blood were collected and stored at -20 $^0$C for measurement of the serum EPO level by ELISA (Research & Development Co.). The assay was performed according to the Benchtop Protocol (n = 30). The coefficient of variation (CV) within the assay for serum EPO (<100 mIU/ml) ranged from 2.84 to 5.22 per cent and for the precision of the method ranged from 4.24 to 8.25 per cent (Quantikine TM *In Vitro* Diagnostic: Human EPO Immunoassay 1997)[8]. For hematologic parameters, 3 ml of blood samples were collected. The ratio of 0.06 ml $K_3$EDTA (0.235 mol/l, 10.5%) to 3 ml of blood was maintained. Samples were stored at room temperature (20-25°C) and then analyzed by a Technicon H*3 RTC. The system was calibrated and operated as recommended in the manufacturer's instructions (Technicon H*3 RTX TM System Operating Guide 1993)[9]. Storage times in anticoagulant before analysis varied from one to three hours. All the experiments were done at room temperature.

The following hematologic parameters i.e., red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), reticulocyte count as percentage (%) and absolute number (#) were used in this study. With the Technicon H*3 technology, the reticulocytes were subdivided into three populations i.e., low, medium and high RNA content depending on the amount of the RNA content. The high RNA content, reported by Technicon H*3 as high-reticulocyte (H-retic), was considered typical for the most immature reticulocytes, while medium-reticulocyte (M-retic) and low-reticulocyte (L-retic) represented the more mature and the most mature reticulocytes, respectively[9]. However, H-retic and M-retic were still considered the immature reticulocyte fraction[10].

**Calculation:**

**1. The reference range of EPO**

To establish the reference range for EPO, we chose to follow the recommendation of the National Committee for Clinical Laboratory Standards (NCCLS)[11] using the reference limit of the 2.5th percentile and 97.5th percentile in 95 per cent of the population observed. The mean value of EPO level was analyzed. Difference between genders was calculated using the unpaired Student *t*' test. $p \leq 0.05$ was considered statistically significant.

**2. The correlation between the EPO level and hematologic parameters**

The mean and standard deviation of the EPO level were determined. For hematologic parameters i.e., red blood cell count, Hb, PCV, reticulocyte count (percentage and absolute number), the mean values and standard deviations were also analyzed. The correlation between the EPO level and these hematologic parameters was determined using Pearson correlation coefficient. The statistical significance of difference between EPO levels and hematologic parameters was evaluated using the two-tailed Student's *t*-test for unpaired data and correlation was considered to have significance at p-value $\leq 0.05$.

## RESULTS

A total of 200 healthy subjects were analyzed, 100 (50%) males and 100 (50%) females, aged between 17 and 60 years, mean age 38.93± 10.20 years. The reference interval of EPO was 2.82-16.68 mU/ml for females (mean = 8.40 mU/ml, range = 1.03-20.40 mU/ml), 1.76-25.29 mU/ml for males (mean = 8.62 mU/ml, range = 0.70-30.50 mU/ml), and 2.21-20.95 mU/ml for the total (mean = 8.51 mU/ml, range = 0.70-30.50 mU/ml). Based on our results, there was no statistically significant difference between the EPO mean of females and males (p=0.751).

Table 1 demonstrated the means (±SD) of EPO and hematologic parameters in females, males and the total. Table 2 showed the correlation between the EPO level and hematologic parameters in females, males and the total. Fig. 1 demonstrates the linear regression of EPO and hematologic parameters.

According to the results in Table 1, there was a statistically significant difference ($p<0.01$) between hematologic parameters i.e., red blood cell count, Hb, PCV and reticulocyte count, among females and males whereas there was no such difference for EPO level regarding the sexes.

**Table 1. The means (± SD) of EPO level and the other hematologic parameters of female, male and total are shown. The mean levels between female and male are considered statistically different when $p \leq 0.01$, Student's *t*-test for unpaired data.**

| Parameters | Female (n = 100) | Male (n = 100) | p-value | Total (n = 200) |
|---|---|---|---|---|
| EPO level (mIU/ml) | 8.40 (± 3.82) | 8.62 (± 5.83) | 0.751 | 8.51 (± 4.93) |
| Age (year) | 37.2 (± 10.32) | 40.65 (± 9.82) | 0.016 | 38.93 (± 10.20) |
| Reticulocyte count (%) | 1.4 (± 0.44) | 1.6 (± 0.45) | 0.005* | 1.5 (± 0.45) |
| L-retic (%) | 84.76 (± 4.83) | 83.46 (± 4.73) | 0.057 | 84.11 (± 4.81) |
| M-retic (%) | 12.17 (± 3.91) | 13.64 (± 3.50) | 0.006* | 12.91 (± 3.77) |
| H-retic (%) | 3.08 (± 1.99) | 3.00 (±1.73) | 0.759 | 3.04 (± 1.86) |
| IFR (H- + M-retic) (%) | 15.25 (± 4.84) | 16.64 (±4.98) | <0.001* | 15.95 (± 4.77) |
| Reticulocyte count (x $10^9$/l) | 61.40 (± 20.84) | 78.51 (± 38.54) | <0.001* | 67.91 (± 22.42) |
| L-retic (x $10^9$/l) | 51.62 (± 16.26) | 64.90 (± 29.25) | <0.001* | 56.59 (± 17.18) |
| M-retic (x $10^9$/l) | 7.83 (± 4.61) | 11.06 (± 7.00) | <0.001* | 9.18 (± 4.90) |
| H-retic (x $10^9$/l) | 1.96 (± 1.63) | 2.62 (± 3.29) | 0.073 | 2.18 (± 1.75) |
| IFR (H-retic+M-retic) (x $10^9$/l) | 9.79 (± 5.85) | 13.68 (± 6.66) | 0.063 | 11.36 (± 6.26) |
| RBC (x $10^{12}$/l) | 4.38 (± 0.32) | 4.91 (± 0.44) | <0.001* | 4.65 (± 0.47) |
| Hb (g/dL) | 12.7 (± 0.66) | 14.4 (± 0.74) | <0.001* | 13.5 (± 1.10) |
| PCV (decimal fraction) | 0.39 (± 0.02) | 0.43 (± 0.02) | <0.001* | 0.41 (± 0.03) |

IFR, Immature fraction of reticulocyte
* Statistical significance at ≤ 0.01

**Table 2. The correlation (data presented as linear regression value, r) between EPO level and the other haematologic parameters in female, male and total.**

| Parameters | Female | Male | Total |
|---|---|---|---|
| EPO level (mIU/ml) | 1.000**h | 1.000** h | 1.000** h |
| Age (year) | 0.111 n | 0.176 n | 0.147* n |
| Reticulocyte count (%) | 0.218* l | 0.178 n | 0.191** n |
| L-retic (%) | -0.204* l | -0.239* l | -0.220**l |
| M-retic (%) | 0.205* l | 0.172 n | 0.180* n |
| H-retic (%) | 0.077 n | 0.250* l | 0.170* n |
| IFR (H-retic+M-retic) (%) | 0.205* l | 0.242* l | 0.221** l |
| Reticulocyte count (x $10^9$/l) | 0.294** l | 0.040 n | 0.061 n |
| L-retic (x $10^9$/l) | 0.282** l | 0.012 n | 0.022 n |
| M-retic (x $10^9$/l) | 0.285** l | 0.101 n | 0.144* n |
| H-retic (x $10^9$/l) | 0.138 n | 0.138 n | 0.161* n |
| IFR (H-retic+M-retic) (x $10^9$/l) | 0.263** l | 0.116 n | 0.158* n |
| RBC (x $10^{12}$/l) | -0.086 n | -0.302** l | -0.171* n |
| Hb (g/dL) | 0.015 n | -0.289** l | -0.098 n |
| PCV (decimal fraction) | -0.014 n | -0.275** l | -0.114 n |

IFR, immature fraction of reticulocyte - (minus), inverse correlation
*, Correlation is significant at the 0.05 level (2-tailed)
**, Correlation is significant at the 0.01 level (2-tailed)
correlation; 0.8-1.0 = h, high correlation
0.5-0.8 = m, moderate correlation
0.2-0.5 = l, low correlation
0.0-0.2 = n, no correlation

There were low correlations between the EPO level and some hematologic parameters (Table 2). There was no corrrelation between the EPO level and age. There were low inverse correlations between the EPO level and the red blood cell count, Hb and PCV levels in male.

## DISCUSSION

Our study demonstrated the EPO reference range, was 2.82-16.68 mU/ml for females, 1.76-25.29 mU/ml for males, and 2.21-20.95 mU/ml for the total, in healthy Thai adults. The normal level of 9-55 mU/ml was reported in a study that measured EPO level by ELISA in 18 normal donors(12). The discrepancy might be due to the differences in the characteristics of the studied population i.e., race, age, gender etc. This was not to mention the number of the population which was much lower than our study. Another study suggested a range of 18-35 mU/ml by radio-immuno assay (RIA)(13).

In healthy adults, it was not surprising that there was a significant difference between females and males in terms of red blood cell count, Hb, PCV and reticulocyte count. There was no such significant difference for EPO level. Our results were in accordance with another report done in normal non-anemic males and females that there was no difference between the EPO level in males and females, no significant correlation between serum EPO level and age and serum EPO level was not correlated to the hemoglobin level(14).

Based on the results in Table 2, the EPO level positively affected the reticulocyte parameters. The higher the EPO level, the more the reticulocyte count increases, especially increasing in the absolute number rather than the percentage change. This could easily be explained since the percentage of the reticulocyte depended on the total number of red blood cells counted irrespective of the individual's condition e.g., anemia, polycytemia. In other words, when the retculocyte count was reported as a percentage, it did not indicate the absolute number of reticulocytes being produced. This information suggested that depicting the reticulocyte count as an absolute number would be more accurate in reflecting eryhtropoiesis since as a percentage it reflected changes in the balance of the production and the destruction of RBC. As reported in other

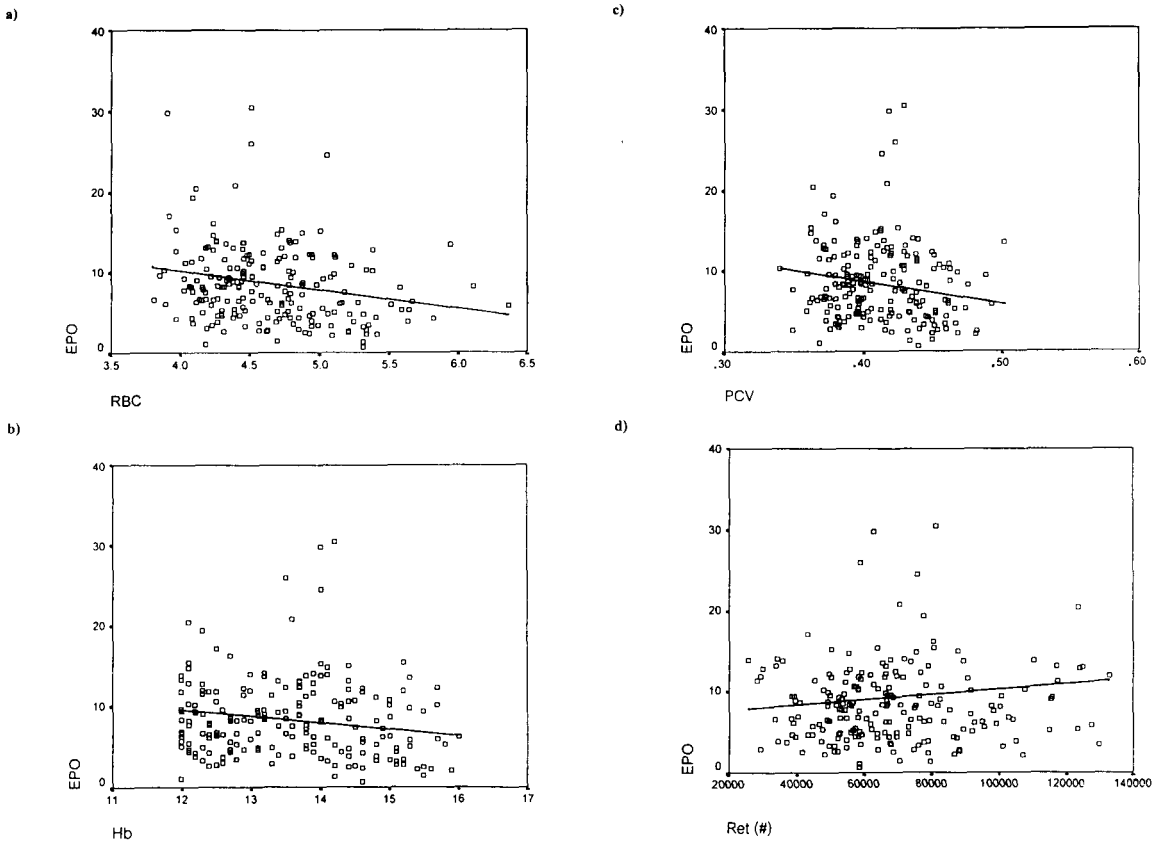


**Fig. 1. Linear regressions of erythropoietin level (EPO) (mU/ml) plotted VS; a) red blood cell count (RBC) (X10$^9$/l); b) hemoglobin (Hb) (g/dl); c) packed cell volume (PCV) (decimal fraction) and d) Reticulocyte (Ret) (absolute number, #)**

studies we also found the correlation between EPO level and the absolute reticulocyte count was absent in males since erythropoiesis in males was not only influenced by the EPO level but also the testosterone level.[15-17] We also investigated the correlation between the EPO level and reticulocyte fractions i.e., the more mature fraction (L-retic) and the immature fractions (H-retic and M-retic). There was no conclusive information in terms of the reticulocyte fractions that could be deduced in this study.

A number of reports demonstrated that the EPO level in healthy adults was not affected by testosterone despite showing that testosterone stimulated erythropoiesis[15-17]. Since we found low inverse correlations between the EPO level and some hematologic parameters i.e., red blood cell count, Hb and PCV in males but not in females, these might reflect the influence of testosterone as one of the many factors controlling the erythropoietic activity. Further investigations are required in order to answer this question.

The inverse correlations seen might suggest that the EPO level and erythropoiesis were part of a negative feedback cycle that controled the number of erythrocytes circulating in the blood. It should also be noted that in the normal physiologic level, EPO production had a direct effect on Hb and PCV, and this relationship is part of a negative-feedback control. In a person with normal kidney function, the level of EPO increased as the Hb decreased[18]. Previous reports done in anemic patients suggested that a proper EPO response could be assessed when the EPO level was assayed together with either Hb or PCV value since the Hb or PCV results correlated well with the EPO level [19-21]. In other words, either the Hb or PCV value could be used to reflect the EPO level in an individual with anemia.

The mechanisms of increasing EPO production during tissue hypoxia which resulted in the increase of erythroid precursors and reticulocyte numbers in response to EPO were interrelated. EPO synthesis as the kidneys' response to hypoxia has been shown to be exponential[3]. In addition, a linear decline in PCV was accompanied by an exponential increase in plasma EPO level in an individuals with a normal capability to produce EPO[22, 23]. As a result, they suggested the use of PCV and reticulocyte count as indicators of the EPO response with a sensitivity of 96 per cent, a specificity of 79 per cent and an overall accuracy of 88 per cent, respectively. These were reported in cancer-related anemic patients having received rHu-EPO. A recent study investigated the capability of hematologic parameters to indicate the response to rHu-EPO in cancer-related chronic anemia[5].

We concluded that the EPO reference range was 2.21-20.95 mU/ml for healthy Thai adults and there were none or low correlations between the EPO level and hematologic parameters in healthy adults. As a result, the studied hematologic parameters could not be used to indicate the level of EPO in healthy adults.

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# ระดับอีริโธพอยอิทินและค่าพารามิเตอร์ต่างๆ ทางโลหิตวิทยาในคนไทย†

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เนื่องจากการควบคุมทางสรีรวิทยาการสร้างเม็ดเลือดแดงเกี่ยวข้องกับระดับอีริโธพอยอิทิน การหาความสัมพันธ์ระหว่างระดับอีริโธพอยอิทินกับค่าพารามิเตอร์ต่างๆทางโลหิตวิทยาซึ่งเป็นการตรวจประจำทางโลหิตวิทยาและมีค่าใช้จ่ายต่ำย่อมจะเป็นประโยชน์ ผู้บริจาคโลหิตจำนวน 200 ราย ที่มีอายุระหว่าง 17-60 ปี แบ่งเป็นเพศชาย 100 ราย และเพศหญิง 100 ราย ถูกเลือกเพื่อการศึกษานี้โดยวิธีสุ่ม การตรวจหาระดับอีริโธพอยอิทินใช้วิธี enzyme linked immunosorbent assay (ELISA) โดยใช้น้ำยาชุดจากบริษัท Research & Development System และตรวจหาค่านับเรติคูโลไซต์ และค่าพารามิเตอร์ต่างๆ ทางโลหิตวิทยาที่เกี่ยวข้องกับเม็ดเลือดแดง โดยเครื่องตรวจวิเคราะห์เซลล์เม็ดเลือดอัตโนมัติ Technicon H*3 RTC ความสัมพันธ์ระหว่างระดับอีริโธพอยอิทินกับค่าพารามิเตอร์ต่างๆ ทางโลหิตวิทยามีค่าอยู่ระหว่าง -0.302 ($P < 0.01$) และ 0.294 ($p < 0.01$) ผลการศึกษานี้พบว่าระดับค่าอ้างอิงของอีริโธพอยอิทินในคนไทยเท่ากับ 2.21-20.95 mU/ml โดยระดับอีริโธพอยอิทินมีความสัมพันธ์กับค่าพารามิเตอร์ต่างๆ ทางโลหิตวิทยาต่ำ และไม่สามารถนำค่าพารามิเตอร์ต่างๆ ทางโลหิตวิทยา มาใช้เพื่อพยากรณ์ระดับอีริโธพอยอิทินในคนปกติได้

**คำสำคัญ** : Erythropoietin, Reference Range, Correlation Study, Hematologic Parameters, Healthy Adults

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