A Multiple Regression Analysis of Hemoglobin Values and Iron Status in Japanese Farmers

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Summary The authors performed multiple regression analysis of hemoglobin values and the parameters related to iron dynamics. The subjects consisted of 37 farmers whose Hb, Ht, GB, SI, %-St, SFR and FEP was examined once a month for one year. 19.2% of the female farmers had anemia of which 73.8% was iron-deficiency anemia, 13.7% being latent iron deficiency. Hb, GB and FEP in males and SI, %-St, SFR, FEP in females revealed clear seasonal variations. Multiple correlation coefficient between Hb and SI, %-St, SFR, FEP as independent variables was significant in females but not in males, and the ratio of contribution (38.7%) was not very high, however, the multiple correlation coefficient was significantly high (0.622). Standard regression coefficients between Hb and SFR, FEP were significantly high (p < 0.01). When Ht and GB were added to these 4 variables, multiple correlation coefficients (male, 0.906; female, 0.957) were remarkably high, which means serum-related variables such as Ht and GB have an undeniably important role as explanatory variables of hemoglobin levels. These standard regression coefficients showed seasonal changes.
Iron-deficiency anemia is probably the most common form of nutritional deficiency in both developing and developed countries (1). It is reported to be the most common cause of anemia, both in general medical practice and in the practice of clinical hematology, and is even alleged to be the most common organic disorder seen in clinical medicine (2). Despite these assertions, relatively few adequate studies have been done on the prevalence of iron deficiency in various populations (3). The most commonly employed screening procedure is a simple blood hemoglobin determination. As measured by this test, anemia was defined by the World Health Organization as having a value below 12 g/dl in nonpregnant women. Such a screening method cannot, by definition, detect latent iron deficiency. Furthermore, inaccuracies are introduced by anemias due to causes other than iron deficiency. Screening by determination of plasma iron and iron-binding capacity obviates some of these inaccuracies. Determination of iron stores is less suited for the study of large groups.

Of total body iron, only a minute portion is found in the plasma, where it is carried while bounded to a transferrin. The remainder is either bound in a porphyrin ring as a part of hemoglobin, myoglobin, or one of the heme enzymes, or is laid aside as storage iron. Ferritin and hemosiderin, the storage forms of iron constitute normally about 30% of the body iron. Therefore, it is important to estimate body iron totally for investigation of causality and dynamics in iron-deficiency anemia (4).

In this study, the authors analyzed the relationships between hemoglobin values and some of the parameters related to iron dynamics, as mentioned above, and their seasonal variations by using multiple linear regression methods (5).

SUBJECTS AND METHODS

The subjects consisted of 37 farmers (17 males and 20 females) ranging in age from 30 to 59 who were mainly engaged in greenhouse agriculture in Kumamoto Prefecture, which is located in the center of Kyushu in southwestern Japan. These subjects were selected from the 154 adults who formed the population of a small village, in consideration of representativeness on the basis of a health examination.

The subjects’ blood values were examined once a month for one year from April 1982 to March 1983. None of the subjects was taken ill during the study periods.

The fasting blood was collected in the morning at 7:30–8:30. The test items concerning anemia were hemoglobin concentration: Hb (cyanmethohemoglobin method); hematocrit: Ht (capillary method using a high speed centrifuge); specific gravity of whole blood: GB (copper sulfate method); serum iron: SI and total iron-

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binding capacity: TIBC (colorimetric method); calculated transferrin saturation: %St; serum ferritin: SFR (immunoradiometric assay); and red cell protoporphyrin: FEP (fluorometric assay).

Multiple linear regression enabled estimation of a least-squares regression equation between a dependent (hemoglobin values) variable and 6 independent variables (Ht, GB, SI, %St, SFR and FEP). The computations were performed on all the data. The equality of regression lines across groups was tested. The multiple correlation coefficient, standard error of an estimated value, standardized and unstandardized regression coefficients, significance of coefficient and $p$ values were calculated (6).

RESULTS

1. Hemoglobin values and iron status

The mean Hb value all through the year was 15.3±0.7 g/dl for males and 13.0±1.2 g/dl for females. The prevalence rate of values below 13.0 g/dl for males was 1.1% and that below 12.0 g/dl for females, 19.2%. As shown in Table 1, 20.6% of the female subjects had abnormal values in one or more variables, which indicate the level of storage iron (7). Out of 42 cases who showed low hemoglobin values, 31 (73.8%) had accompanying iron deficiency. Eventually 14.1% of the female subjects were found to have iron deficiency anemia and 5.1% had low hemoglobin values without iron deficiency. Furthermore, 13.7% of the female subjects had latent iron deficiency in whom about 30% showed abnormal values of SFR alone. Moreover, 6.9% of the female subjects showed abnormal values of FEP alone.

Table 1. Prevalence rate of abnormal values in the haematological variables (Female).

| Normal 133 (60.4%) | Abnormal 87 (39.6%) | Total 220 |
|--------------------|---------------------|----------|
| SFR + FEP + %St + Hb | 21(9.6%) | 31(14.1%) | 42(19.2%) |
| SFR + %St + Hb | 4(1.8) | | |
| SFR + FEP + Hb | 3(1.4) | | |
| SFR + Hb | 3(1.4) | | |
| FEP + Hb | 3(1.4) | | |
| Hb | 8(3.7) | | |
| SFR + FEP + %St | 4(1.8) | | |
| FEP + %St | 3(1.4) | | |
| SFR + %St | 3(1.4) | | |
| SFR + FEP | 2(0.9) | | |
| %St | 8(3.7) | | 18(8.2) |
| SFR | 10(4.6) | | |
| FEP | 15(6.9) | | |

Criteria of abnormality: Hb < 12.0 g/dl, %St < 20%, SFR < 20 ng/ml, FEP > 70 µg/dl·PCV

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2. Seasonal variations of hemoglobin variables

Table 2 shows the mean value of 7 variables, measured all through the year. Hb, GB and FEP in males, and SI, %St, SFR and FEP in females revealed remarkable seasonal variations \( p < 0.01 \) based on calculation of \( F \)-value and \( P \)-value. Figure 1 shows the seasonal variations of Hb, %St, SFR and FEP. Hemoglobin values decreased in summer and increased in winter as previously reported (6). On the other hand, %St and FEP increased from summer to autumn, and then decreased from winter to spring in contrast to Hb. Sex differences in the variation of SFR and %St were observed; however, this was not the case with Hb and FEP.

Table 3 shows the correlation matrix among monthly measured values of hemoglobin variables. The correlation coefficients of Hb, SFR and FEP were high; however, those of SI and %St showed large variations.

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**Table 2. Mean value of 6 haematological values all through the year.**

|       | Male         | Female       |
|-------|--------------|--------------|
| Hb    | 15.30 ± 0.68 | 13.01 ± 1.18 |
| Ht    | 46.05 ± 2.36 | 39.59 ± 3.03 |
| GB    | 1.0572 ± 0.0015 | 1.0525 ± 0.0026 |
| SI    | 106.80 ± 23.14 | 94.31 ± 30.10 |
| %St   | 37.19 ± 9.11  | 29.64 ± 9.05  |
| SFR   | 113.76 ± 88.55 | 44.30 ± 30.26 |
| FEP   | 33.92 ± 10.31 | 54.86 ± 24.43 |

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![Fig. 1. Seasonal variation of haematological values. (A): male. (B): female.](image-url)
3. Relationships between hemoglobin and the other variables

As shown in Table 4, the correlation coefficients between almost all variables were significantly high in females; however, those among Hb, Ht, GB were significant in males.

The results of multiple regression analysis between hemoglobin values and the other variables are shown in Table 5. With SI, %-St, SFR and FEP as independent variables, the multiple correlation coefficient was not significant (0.259) in male subjects, and in female subjects the ratio of contribution (38.7%) was not very high; however, the multiple correlation coefficient was significantly high (0.622). In this case, standard regression coefficients between Hb, SFR, and FEP were significantly high ($p < 0.01$), which means the latter two variables contributed the most to those parts of the Hb values which could be explained by iron-related variables. The results suggest that Hb levels can not be explained by iron-related variables in male subjects and that iron-related values could explain about 40% of Hb levels in female subjects. The ratio of contribution to hemoglobin values in the iron-related variables may depend on the value of hemoglobin levels.

When Ht and GB were added to the previous 4 parameters, multiple correlation coefficients (male, 0.906; female, 0.957) were remarkably high. These coefficients do not necessarily show the weight of Ht and GB as explanatory variables of hemoglobin values, but it is undeniable that serum-related variables such as Ht and GB play important roles as explanatory variables of hemoglobin values.

Figure 2 shows the correlational diagram between the observed Hb values and the predicted Hb values estimated from the previous two cases of multiple correlation equations.

Figure 3 shows the seasonal variations of the ratio of contribution to hemoglobin levels in Ht, GB, SI, %-St, SFR and FEP according to the standard regression coefficients based on the same method as shown in Table 4. In female subjects, the ratio of contribution in SI, %-St, SFR and FEP was highest in winter (53.3%) and lowest in summer and autumn (30–33%). These seasonal variations seem to be greatly influenced by FEP values. On the other hand, Hb values were greatly influenced by Ht and GB values in summer and autumn. It is suggested that %-St and SFR in spring and FEP in winter play important roles as explanatory variables in the prediction of Hb values.

DISCUSSION

Anemia is the end result of severe deficiency, however, the Hb concentration and/or Ht are relatively insensitive indices of milder degrees of iron depletion that are common in the female population in Japan. Wherever possible, therefore, employment of other parameters of iron status in addition to the measurement of Hb and Ht is highly desirable (8). Iron-replete adults not only have normal Hb
Table 3. Correlation matrix of monthly variation in haematological values (Female).

|     | Apr.   | May    | Jun     | Jul     | Aug     | Sep     | Oct     | Dec     | Jan     | Feb     | Mar     |
|-----|--------|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Hb  | 1.000  | .000   |         |         |         |         |         |         |         |         |         |
|     | 0.847* | .000   | .000    | .000    | .000    | .000    | .000    | .000    | .000    | .000    | .000    |
|     | .079*  | .026*  | .090    | .042    | .012    | .000    | .000    | .000    | .000    | .000    | .000    |
|     | .066*  | .068*  | .059*   | .053*   | .026*   | .000    | .000    | .000    | .000    | .000    | .000    |
|     | .081*  | .086*  | .092*   | .081*   | .000    | .000    | .000    | .000    | .000    | .000    | .000    |
|     | .079*  | .073*  | .093*   | .082*   | .034*   | .035*   | .033*   | .036*   | .034*   | .034*   | .034*   |
|     | .067*  | .065*  | .070*   | .067*   | .033*   | .034*   | .033*   | .034*   | .034*   | .034*   | .034*   |
|     | .064*  | .057*  | .074*   | .054*   | .029*   | .030*   | .030*   | .030*   | .030*   | .030*   | .030*   |
|     | .059*  | .043*  | .060*   | .065*   | .056*   | .056*   | .056*   | .056*   | .056*   | .056*   | .056*   |
|     | .067*  | .044*  | .060*   | .065*   | .057*   | .058*   | .058*   | .058*   | .058*   | .058*   | .058*   |
|     |       |       |         |         |         |         |         |         |         |         |         |

**Z-St**

|     | Apr.   | May    | Jun     | Jul     | Aug     | Sep     | Oct     | Dec     | Jan     | Feb     | Mar     |
|-----|--------|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
|     | 1.000  | .000   | .000    | .000    | .000    | .000    | .000    | .000    | .000    | .000    | .000    |
|     | .295   | .000   | .000    | .000    | .000    | .000    | .000    | .000    | .000    | .000    | .000    |
|     | .623*  | .014*  | .042*   | .044*   | .000    | .000    | .000    | .000    | .000    | .000    | .000    |
|     | .423*  | .016*  | .016    | .016    | .000    | .000    | .000    | .000    | .000    | .000    | .000    |
|     | .557*  | .032*  | .069*   | .016*   | .000    | .000    | .000    | .000    | .000    | .000    | .000    |
|     | .356   | .026*  | .071*   | .071*   | .000    | .000    | .000    | .000    | .000    | .000    | .000    |
|     | .350*  | .049*  | .050*   | .050*   | .000    | .000    | .000    | .000    | .000    | .000    | .000    |
|     | .151   | .019*  | .185*   | .285*   | .033*   | .034*   | .034*   | .034*   | .034*   | .034*   | .034*   |
|     | .008*  | .055*  | .071*   | .071*   | .034*   | .034*   | .034*   | .034*   | .034*   | .034*   | .034*   |
|     | .013*  | .015*  | .031*   | .031*   | .031*   | .031*   | .031*   | .031*   | .031*   | .031*   | .031*   |
|     | .043*  | .053*  | .069*   | .069*   | .053*   | .053*   | .053*   | .053*   | .053*   | .053*   | .053*   |
|     |       |       |         |         |         |         |         |         |         |         |         |

**Spring Summer Autumn Winter**

|     | 1.000  | .000   | .000    | .000    | .000    | .000    | .000    | .000    | .000    | .000    | .000    |
|     | .816*  | .000   | .000    | .000    | .000    | .000    | .000    | .000    | .000    | .000    | .000    |
|     | .716*  | .026*  | .000    | .000    | .000    | .000    | .000    | .000    | .000    | .000    | .000    |
|     | .479*  | .014*  | .000    | .000    | .000    | .000    | .000    | .000    | .000    | .000    | .000    |

NOTE: Spring Mar. Apr. May  Summer Jun Jul Aug. Autumn Sep. Oct. Winter Dec. Jan. Feb.

* p<0.05
| SFR   | Apr. | May | Jun. | Jul. | Aug. | Sep. | Oct. | Dec. | Jan. | Feb. | Mar. |
|-------|------|-----|------|------|------|------|------|------|------|------|------|
|       | 1.000 |     |      |      |      |      |      |      |      |      |      |
|       | 0.856* | 1.000 |      |      |      |      |      |      |      |      |      |
|       | 0.936* | 0.915* | 1.000 |      |      |      |      |      |      |      |      |
|       | 0.919* | 0.934* | 0.981* | 1.000 |      |      |      |      |      |      |      |
|       | 0.874* | 0.945* | 0.931* | 0.936* | 1.000 |      |      |      |      |      |      |
|       | 0.896* | 0.902* | 0.943* | 0.950* | 0.919* | 1.000 |      |      |      |      |      |
|       | 0.817* | 0.739* | 0.803* | 0.803* | 0.789* | 0.806* | 1.000 |      |      |      |      |
|       | 0.878* | 0.791* | 0.887* | 0.869* | 0.773* | 0.876* | 0.837* | 1.000 |      |      |      |
|       | 0.811* | 0.757* | 0.868* | 0.828* | 0.731* | 0.865* | 0.805* | 0.955* | 1.000 |      |      |
|       | 0.922* | 0.837* | 0.945* | 0.937* | 0.844* | 0.850* | 0.800* | 0.878* | 0.817* | 1.000 |      |
|       | 0.889* | 0.918* | 0.944* | 0.955* | 0.935* | 0.906* | 0.755* | 0.812* | 0.754* | 0.878* | 1.000 |

|       | Spring | Summer | Autumn | Winter |
|-------|--------|--------|--------|--------|
|       | 1.000  | 0.977* | 1.000  |        |
|       | 0.885* | 0.873* | 1.000  |        |
|       | 0.974* | 0.952* | 0.924* | 1.000  |

| FEP   | Apr. | May | Jun. | Jul. | Aug. | Sep. | Oct. | Dec. | Jan. | Feb. | Mar. |
|-------|------|-----|------|------|------|------|------|------|------|------|------|
|       | 1.000 |     |      |      |      |      |      |      |      |      |      |
|       | 0.750* | 1.000 |      |      |      |      |      |      |      |      |      |
|       | 0.601* | 0.962* | 1.000 |      |      |      |      |      |      |      |      |
|       | 0.714* | 0.922* | 0.921* | 1.000 |      |      |      |      |      |      |      |
|       | 0.530* | 0.735* | 0.738* | 0.796* | 1.000 |      |      |      |      |      |      |
|       | 0.802* | 0.964* | 0.945* | 0.943* | 0.740* | 1.000 |      |      |      |      |      |
|       | 0.769* | 0.903* | 0.889* | 0.910* | 0.700* | 0.959* | 1.000 |      |      |      |      |
|       | 0.863* | 0.855* | 0.822* | 0.858* | 0.655* | 0.896* | 0.861* | 1.000 |      |      |      |
|       | 0.775* | 0.681* | 0.634* | 0.768* | 0.486* | 0.753* | 0.727* | 0.873* | 1.000 |      |      |
|       | 0.846* | 0.662* | 0.606* | 0.698* | 0.416* | 0.716* | 0.669* | 0.863* | 0.952* | 1.000 |      |
|       | 0.759* | 0.681* | 0.648* | 0.766* | 0.485* | 0.750* | 0.725* | 0.886* | 0.975* | 0.955* | 1.000 |

|       | Spring | Summer | Autumn | Winter |
|-------|--------|--------|--------|--------|
|       | 1.000  | 0.894* | 1.000  |        |
|       | 0.964* | 0.921* | 1.000  |        |
|       | 0.793* | 0.708* | 0.830* | 1.000  |

* p < 0.05
concentrations but also have iron stores of 500 to 1,000 mg. The size of these stores has been assessed in the past through a variety of invasive techniques, such as histological examination of bone marrow smears for the presence of stainable iron. More recently, however, using immunological methods, it has been shown that small amounts of ferritin are present in the serum, which reflect body iron stores. SFR measurements, therefore, provide a convenient method of assessing the amount of storage iron which can be fairly readily applied to population groups (9).

If erythropoietic precursors are deprived of their normal iron supply, not all the available protoporphyrin will be formed into heme and as a result there will be a concomitant rise in FEP (10). At the same time, any impairment in Hb production eventually will lead to a decrease in Hb concentration to levels below the lowest observed in normal subjects.

The initial effect of a normal individual being subjected to a continuing negative iron balance is a gradual decrease in SFR. With a continued negative iron balance, the probability will increase that either the %-St or FEP or both will fall outside their normal range. While the Hb will also decrease during this phase, the change may go unrecognized because of the broad range of Hb values in a normal population.

International Nutritional Anemia Consultation Groups recommended that ideally, all of these parameters be used when assessing the iron status of a population, but that the most important parameters were Hb and SFR which

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Table 4. Correlation matrix among haematological values.

|       | Hb   | Ht   | GB   | SI   | %-St | SFR  | FEP  |
|-------|------|------|------|------|------|------|------|
| Male  |      |      |      |      |      |      |      |
| Hb    | 1.000|      |      |      |      |      |      |
| Ht    | 0.868*| 1.000|      |      |      |      |      |
| GB    | 0.829*| 0.845*| 1.000|      |      |      |      |
| SI    | -0.030| 0.135| 0.123| 1.000|      |      |      |
| %-St  | -0.098| -0.080| -0.099| 0.714*| 1.000|      |      |
| SFR   | 0.165| 0.233| 0.070| 0.142| 0.349*| 1.000|      |
| FEP   | -0.092| -0.218| -0.051| -0.107| -0.048| -0.136| 1.000|
| Female|      |      |      |      |      |      |      |
| Hb    | 1.000|      |      |      |      |      |      |
| Ht    | 0.910*| 1.000|      |      |      |      |      |
| GB    | 0.917*| 0.877*| 1.000|      |      |      |      |
| SI    | 0.449*| 0.410*| 0.372*| 1.000|      |      |      |
| %-St  | 0.437*| 0.302*| 0.361*| 0.738*| 1.000|      |      |
| SFR   | 0.398*| 0.265*| 0.347*| 0.213| 0.383*| 1.000|      |
| FEP   | -0.534*| -0.440*| -0.447*| -0.642*| -0.472*| -0.208*| 1.000|

* p<0.05
monitor both ends of the spectrum of iron status. Epidemiologic studies have, however, indicated that this is not so in subjects with mild iron deficiency. This is probably due to the fact that the analytic and biologic variability of the laboratory tests may be relatively large in relation to the degree of deviation from normal. Because of this, it is necessary to use the tests in an integrated way when attempting to define the iron nutritional status of population groups. In such studies it has been customary to determine the percentage of subjects with values outside the normal range (II). In this sense, the authors investigated the multiple regres-

Table 5. Multiple regression analysis between Hb and the haematological values.

|          | Male                                      | Female                                   |
|----------|-------------------------------------------|------------------------------------------|
| Hb       | SI, % St, SFR, FEP                        | SI, % St, SFR, FEP                       |
| Multiple correlation $R=0.259$ (Ratio of contribution: 6.7%), $p=0.35$ | Multiple correlation $R=0.622$ (Ratio of contribution: 38.7%), $p<0.01$ |
| Multiple regression equation (Hb) | Multiple regression equation (Hb) |
| $Hb = 15.460 + 0.004 \cdot SI - 0.021 \cdot %St + 0.004 \cdot SFR - 0.004 \cdot FEP$ | $Hb = 12.930 + 0.003 \cdot SI + 0.013 \cdot %St + 0.001 \cdot SFR - 0.019 \cdot FEP$ |
| Standard regression coefficient | Standard regression coefficient |
| SI: $0.123\ (p=0.49)$ | SI: $0.069\ (p=0.66)$ |
| % St: $-0.271\ (p=0.15)$ | % St: $0.103\ (p=0.47)$ |
| SFR: $0.234\ (p<0.10)$ | SFR: $0.264\ (p<0.01)$ |
| FEP: $-0.060\ (p=0.63)$ | FEP: $-0.386\ (p<0.01)$ |
| Hb: Ht, GB, SI, % St, SFR, FEP | Hb: Ht, GB, % St, SFR, FEP |
| Multiple correlation $R=0.906$ (Ratio of contribution: 82.0%), $p<0.01$ | Multiple correlation $R=0.958$ (Ratio of contribution: 91.7%), $p<0.01$ |
| Multiple regression equation (Hb) | Multiple regression equation (Hb) |
| $Hb = -1.327 + 0.210 \cdot Ht + 0.126 \cdot GB - 0.001 \cdot SI + 0.013 \cdot %St - 0.001 \cdot SFR + 0.003 \cdot FEP$ | $Hb = -4.072 + 0.189 \cdot Ht + 0.184 \cdot GB - 0.003 \cdot SI + 0.016 \cdot %St + 0.003 \cdot SFR - 0.006 \cdot FEP$ |
| Standard regression coefficient | Standard regression coefficient |
| Ht: $0.706\ (p<0.01)$ | Ht: $0.484\ (p<0.01)$ |
| GB: $0.271\ (p<0.05)$ | GB: $0.398\ (p<0.01)$ |
| SI: $-0.266\ (p<0.01)$ | SI: $-0.081\ (p<0.01)$ |
| % St: $0.164\ (p<0.10)$ | % St: $0.119\ (p<0.05)$ |
| SFR: $-0.031\ (p=0.64)$ | SFR: $0.078\ (p<0.05)$ |
| FEP: $0.051\ (p=0.39)$ | FEP: $-0.122\ (p<0.01)$ |
Fig. 2. Correlation diagram between predicted value and observed value of Hb (female). (A): predicted value by the multiple regression equation involved by SI, %-St, SFR and FEP. (B): predicted value by the multiple regression equation involved by Ht, GB, SI, %-St, SFR and FEP.

Fig. 3. Seasonal variation of the contribution ratio of haematological values for Hb.

Regression analysis between the Hb values and the iron status by using monthly data obtained from the same subjects. Yoshino et al. reported that correlation coefficients obtained from the pairs of logarithms of the parameters Hb, Ht, %-St, SFR and

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FEP were highly significant; however, there were problems with differences in the criteria of these parameters (12). In particular, although the value of the FEP/Hb ratio for the lower limit of iron deficiency or average value of SFR in normal adults did not correspond with the results cited in the literature (13, 14), the presence of close correlations in these two parameters as against the others should be evaluated as a marked characteristic in the estimation of iron status. From the results of this study, the authors intend to emphasize the important role of SFR and FEP. It is also suggested that not only iron status but also serum components such as Ht should be noted when the Hb values are estimated, particularly in the summer season, as Furuya reported in the case of pregnant women (15).

The seasonal variation of Hb values is well known, but few reports have been concerned with the seasonal variation of the other variables (%-St, SFR, FEP). The authors analyzed the multiple regression coefficients using material obtained every month to clarify the complex interrelationships among these parameters in all seasons. It is suggested that %-St and SFR in spring, Ht and GB in summer and autumn and FEP in winter are important regulatory and explanatory variables for prediction of Hb values. Seasonal changes in human activities may also account for variation in the requirement of nutrients such as iron. In the future, the authors intend to perform an epidemiological analysis to interpret these seasonal variations.

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