Correlation between Hemoglobin Concentration and Absolute Reticulocyte count of adolescent female iron deficient patients at Colombo north teaching hospital.

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Abstract:-Iron deficiency can be identified as the most common micro-nutrient disorder and cause of anemia. Most frequently occurred in children under the age of 5 years, females of childbearing age and pregnant women. Study population comprised of untreated female patients, between 15-35 years of age (n=111) with Iron deficiency due to nutritional deficiency; from Thalassemia unit, Colombo North teaching hospital. In order to generate a comparison, of the correlations in a healthy person and an Iron deficient patient, a control population (n=60) were selected according age and gender matched patient population. The Thalassemia unit was chosen, as those patients who could make a major effect on hemoglobin levels, could be eliminated. Blood from the selected patients were collected for Full blood count analysis with Reticulocyte count and Serum Ferritin analysis. The Pearson's moment correlation of coefficient (r) of patient population (n=111); between Hb and Abs.Retic is r = - 0.432, a moderate negative correlation (t-test: P=0.000 < 0.01), between Hb and S. Ferritin r = 0.570, a strong positive correlation (t-test: P=0.000 < 0.01), between Abs.Retic count and S. Ferritin r= -0.268, a weak negative correlation (t-test: P=0.000 < 0.01). There were no correlations between parameters of control population. The Hb sub-group 9- 9.9 g/dL of patient population contributed to the significant strong negative correlation (t-test: r=-0.717; P=0.000<0.05) among all the groups. The results of our study shows that the body starts to present iron deficiency (ID) features (Microcytosis, Hypochromasia) in blood picture below Hb value 11.1 g/dL level. Although, above the 11.1 g/dL of Hb the ID features in blood picture is not prominent there are symptoms of ID. At the Hb range 9 – 9.9 g/dL the Abs.Retic count increases by about 6.804 x 10^{10}/L which could be identified as an attempt of compensating the bone marrow in reduction of RBC production, in Iron deficiency anemia.

Keywords: Iron deficiency anemia, Serum Ferritin, Absolute Reticulocyte count, Correlation, ID symptoms

Introduction:

Iron deficiency can be identified as the most common micro-nutrient disorder and cause of anemia. Most frequently occurred in children under the age of 5 years, females of childbearing age and pregnant women. Nutritional iron deficiency occurs when bio availability of iron is insufficient in dietary supplies to meet the body's requirement (Lynch, 2011). Iron deficiency is the most common nutritional deficiency in the world, and it is a global health problem (Camaschella, 2015; Haas et al., 2001). It is a condition in which the mobilizable iron stores are absent which results in compromised iron supply to tissues including erythrocytes. Pallor fatigue and dyspnea are the most common symptoms of anemia (Iron deficiency anemia, 2001; Dallal et al., 2016).

Sri Lanka too is heavily burdened by the problem of anemia, mostly due to nutritional

deficiency of iron. According to a study done by the medical research institute Sri Lanka in 2001 using a sub sample of 2000 population from the District Hospital System, prevalence of anemia among children age 6 -59 months was 32.6%, prevalence of anemia among non-pregnant women age 15- 49 years was 34.1 % and prevalence of anemia among pregnant women age 15 - 49 was 39.1% (Demographic & Health Survey, 2007). According to the host factors: age, gender, physiological, pathological, socio economic and environmental conditions the prevalence of iron deficiency may vary greatly.

The purpose of this study was to find an association between Hb and Absolute reticulocyte count in patients those who are newly diagnosed of Iron deficiency caused by nutrient deficiency, and to compare the statistical correlations in patient and control populations.

Methodology:

Hundred and seventy one adolescent girls and women of childbearing age (15 to 35 years) were enrolled for the study. The participants who were attending the Thalassemia prevention program, CNTH within the time frame of our data collection were selected by their serum ferritin values, confirmed by the Consultant haematologist CNTH. Individuals with serum ferritin value <20 ng/mL were selected as the patient population (n=111) and individuals with serum ferritin values >20 ng/mL were selected as control population (n=60) based on the study population selection criteria of previous study (Thoradeniya et al, 2005). Patients with ID symptoms, and have been excluded for Thalassemia by the Thalassemia Prevention program, CNTH were identified. Written consent were obtained, ensuring the willingness to participate in the research. Blood samples were collected by the nursing staff of the selected individuals. Questions regarding dietery intake of participants, food habits, nutritional practices, knowledge about ID, ID symptoms if any shown and menstrual problems were queried and certain background knowledge about the patients were obtained.

Individuals with a normal healthy Hb concentration, which have been excluded of Thalassemia by the Thalassemia Prevention program, CNTH, were also selected and above mentioned procedures of obtaining consent and collection of blood samples were done similarly to the patient group.

All the samples from patients and controls were checked for visible hemolysis prior to performing the tests, by holding each sample against a clear white color background.

The tests were carried out within 4 hours of sample collection. The samples for Full blood count with reticulocyte count were analysed in Mindray BC6800 fully automated analyser in CDR mode. Hemoglobin estimation by Colorimetry and RBC estimation by Flowcytometry. The samples for serum ferritin were analysed in VITROS 3600 fully automated immunodiagnostic analyser by immunodiagnostic methods. A manual reticulocyte count analysis was performed for 50 randomly selected samples for confirmation of the automated values. A blood picture analysis was performed for samples with Hb < 10.00 g/dL (14 samples) and 36 more random samples to confirm the exclusion of other anemias.

All data analysis was done using IBM SPSS software version 20 and Microsoft office Excel 2010 software.The statistical analysis that was used in the study was Pearson's correlation coefficient.

Results:

Patient Hb values varied from 6.6 to 14.4 g/dL and Hb values of the control population used in the study vary from 12.1 to 15.1 g/dL Ferritin values vary from 3.81 to 19.9 ng/dL in patient population and Ferritin values of the

healthy control population vary from 20.9 to 84.5 ng/dL. Out of the 111 patients 62 patients have a higher Hb value than 11.9 g/dL (according to WHO criteria < 11.9g/dL Hb, defines as anemic.) but they have low serum ferritin values which indicates them as iron deficient but not anemic. They represent 55.85% of whole patient population that strongly indicates even patients with high Hb values can be iron deficient with symptoms. 44.15% of the patient population have IDA. Their Abs.Retic count vary from 1.5 to $11.1X10^{10}$/L in the patient population.

The Pearson's moment correlation of coefficient (r) value between Hb and Abs.Retic of the patient population (n=111) is - 0.432, which indicates a moderate negative correlation between parameters. The 2 parameters showed a significant statistical difference (t-test: P=0.000 < 0.01).

The Pearson's moment correlation of coefficient between Hb and S. Ferritin (r) value of the patient population is 0.570 which indicates a strong positive correlation between Abs.Retic count and S. Ferritin. The parameters showed a significant statistical difference (t-test: P=0.000 < 0.01).

The Pearson's moment correlation of coefficient between Abs.Retic count and S. Ferritin (r) value of the patient population is -0.268 which indicates a weak negative correlation between parameters. The parameters showed a significant statistical difference (t-test: P=0.000 < 0.01).

None of the correlations showed significant correlations (t test: p value > 0.01) in the control population (n=60). Since the Hb and Ab. Retic count parameters indicated a negative correlation the Hb levels were further grouped according to the WHO criteria for anemia. According to the WHO criteria for non-pregnant women the groups are designed as Hb > 11.9 g/dL; 11.0 < Hb > 11.9 g/dL; 8.0 < Hb > 10.9 g/dL; Hb < 8.0 g/dL. The data were re-analysed by Pearson coefficient correlation to identify which group specifically gives the negative correlation. However, there was no correlation in above groups.

The patients were sub-grouped by reducing the width of the range and 7 subgroups were prepared.

The only significant correlation was obtained in 9.0 to 9.9 g/dL Hb group and it was a strong negative correlation (r= -0.717). There were no correlations observed in any other sub-group. In our patient population we observed that the majority (56%) had normal Hb values (Hb > 11.9 g/dL – non anemic) but low iron stores (serum ferritin < 20 ng/dL) suggesting cellular iron deficiency and iron depletion as indicated in Allen et al. (2017).

Discussion:

Our most significant finding in the ID patient population, is the Pearson's moment correlation of coefficient (r) value between Hb and Abs.Retic of the Patient population (n=111) was - 0.432, which indicates a moderate negative correlation between two parameters fulfils our general objective. Since we achieved a significant correlation and also our Hb range width is comparatively large the range was further divided into groups as shown in Table 4. During the analysis we observed that Hb group 9 - 9.9 g/dL was the only group that contributed to the significant correlation (t-test: r=-0.717; P=0.000<0.05) among all the groups. Such finding has not revealed in the literature to our knowledge. The most probable reason as revealed in literature that nutrient deficiencies specially iron, which is a major necessity for RBC production, decreases RBC production hence the reticulocyte count also decreases, resulting in reticulocytopenia (Thurnham and Northrop-Clewes, 2013).

Conclusion:

But as our finding indicates one level of Hb in ID, shows an increased reticulocyte count, which indicates reticulocytosis which may be

due to the bone marrows attempt of trying to compensate the loss of RBC. In the higher Hb levels (>10 g/dL) and very low Hb levels (< 8.9 g/dL) this compensation process and reticulocytosis cannot be observed.

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