A STUDY OF DIFFERENTIAL LEUCOCYTE COUNT IN DIFFERENT GRADES OF ANAEMIA

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ARTICLE INFO

Keywords:
Anaemia
DLC
Hb
NLR

ABSTRACT

Background and objective: Anaemia is the most common nutritional disorder in the world and iron deficiency is implicated in a majority of cases. Anaemia is associated with hypoxia and ischaemia and increased morbidity and mortality. It may be possible that ischaemia associated with anaemia may alter the leucocyte count. White blood cell count is regarded to be an independent risk factor for coronary heart disease, stroke, vascular disease and total morbidity and mortality. WBC count has prognostic importance for both short term and long term survival. Iron deficiency is associated with impairment of cell mediated immunity and the bactericidal activity of neutrophils, thereby increasing the susceptibility to infections. Iron deficiency might play an important role in defence mechanism. The present study is carried out to assess the leucocyte profile in cases of anaemia, and thus early intervention would prevent from facing the possible complications in future.

Material and Methods: Study group comprised of 50 subjects each in control, mild, moderate and severe anaemia. 2 ml of venous blood was drawn and mixed with ethylene diamine tetra-acetic acid (EDTA). The haemoglobin (Hb) and differential leucocyte count (DLC) were measured using autoanalyser.

Results: There was significant increase in neutrophil and lymphocyte percentage in all grades of anaemia. There was also significant decrease in monocyte and eosinophil percentage in all grades of anaemia along with decrease in NLR.

Conclusion: These results suggest that non specific immunity, humoral, cell-mediated and the activity of cytokines which have crucial roles in various steps of immunogenic mechanisms are influenced by the nutritional deficiency.
established fact that many factors are associated with coronary heart disease and their measurements may allow estimation of risk of ischemic events[7].

One such risk factor may be the role played by WBC, particularly the neutrophils assessed most simply by WBC count in peripheral blood[6,7,8]. Recently, neutrophil to lymphocyte ratio [NLR] has emerged as a useful inflammatory index in critically ill patients and ischemic heart disease [IHD][9].

The present study is carried out to assess the leukocyte profile in cases of anemia, and early intervention would prevent from facing the possible complications in future.

MATERIALS AND METHODS:

Source of data

Patients visiting Navodaya Medical College, Hospital and Research Center, Raichur. 50 subjects in each group after classification and categorizing anemia, according to WHO as mentioned below, along with 50 subjects as controls after age and sex matched were selected.

Mild anemia: Hemoglobin of 11 - 11.9 gm/dl in adult female
Hemoglobin of 12 - 12.9 gm/dl in adult male

Moderate anaemia: Hemoglobin of 8 - 10.9 gm/dl in adult female
Hemoglobin of 9 - 11.9 gm/dl in adult male

Severe anemia: Hemoglobin < 8 gm/dl in adult female
Hemoglobin < 9 gm/dl in adult male

Control Group: Hemoglobin >12 gm/dl in adult female
Hemoglobin >13 gm/dl in adult male

Inclusion criteria: Male and female patients in the age group of 20-40
- Male patients having Hemoglobin <12 gm/dl
- Female patients having Hemoglobin <11 gm/dl

Exclusion criteria:
- Severe anemia patients in congestive cardiac failure patients with coexisting morbid conditions like leukemia and other cancerous conditions
- Congenital heart disease
- Diabetes mellitus, hypertension, inflammatory diseases, allergic disorders
- Pregnancy, lactation, dysfunctional uterine bleeding, acute hemorrhagic condition
- Smoker and alcoholics.

Informed consent was obtained from each subject. The study was approved by Ethical Committee, Navodaya Medical College, Raichur. From all the subjects 2ml of venous blood was drawn and anticoagulated using (EDTA). The haemoglobin concentration (Hb%), differential leucocyte count (DLC) were measured using hematology autoanalyser Sysmex KX 21.

The KX-21 employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block using the DC detection method. The Hb detector block measures the hemoglobin concentration using the non-cyanide hemoglobin method.

Statistical methods

Descriptive statistics such as mean, SD, percentage etc was used to present the data. All the Data were expressed in mean ± SD. The significance of difference among the groups was assessed by analysis of variance (ANOVA) followed by post hoc Tukey-Kramer Multiple Comparisons Test for normally distributed data and Friedman test followed by post hoc t test for non-normally distributed data using SPPS v16.0. Comparison between control and anemia groups was done using student’s t-test. A two-tailed p-value less than 0.05 were considered as significant.

RESULT:

Baseline parameters;

The mean age group of the control was 31.84 ± 5.14
The mean age group of the subjects with mild, moderate and severe anemia was 31.46 ± 9.58, 27.70 ± 5.17 and 31.48 ± 5.69 respectively. There was no statistically significant difference in age between two groups (p<0.14).

The male to female ratio was 25/25 in controls and in moderate anaemia, whereas in mild anaemia it was 22/28 and in severe anaemia it was 27/23

Anthropometric parameters:

The mean BMI of controls was 22.73 ± 2.01
The mean BMI of subjects with mild, moderate and severe anemia was 23.02 ± 2.41, 23.12 ± 1.32 and 21.81 ± 2.54 respectively. There was no statistically significant difference in BMI between the two groups (p<0.14).

Comparison of Hemoglobin and DLC between controls and anemic subjects:

The mean ± SD of Hb concentration in anemic and controls group was 9.78 ± 2.07 and 13.44 ± 0.82 respectively. There was decrease in Hb concentration in anemic group compared to controls and this difference was statistically significant with p value of <0.0001.
DISCUSSION

Iron deficiency is one of the most common preventable nutritional deficiencies in developed and developing countries. Iron deficiency anemia can cause irritability, headache, and fatigue that change social behavior and impair the ability of adults to do physical work. An increased susceptibility to infections has been reported in some nutritional anemia patients, the etiology of which is not well-known. Some authors have suggested that altered levels of some interleukins (IL) and cytokines (e.g. IL-2, IL-1, IL-6, TNF-α, IL-4, IL-12p40, IFN-γ, and IL-10) might lead to immune system impairment in iron deficient anemic patients. In addition, it has been suggested that altered cell marker expression may contribute to reduced leucocyte proliferation during iron deficiency [10].

Deficiency of iron and zinc are well documented to impair immune function in experimental animals and to the extent studied, in humans as well. Reported immune defects in iron deficiency include decreased cell-mediated immunity, mitogen responsiveness and natural-killer cell activity. Neutrophil phagocytosis and B lymphocyte function are reported to be generally intact, but lymphocyte bactericidal activity is decreased [11].

This study carried out in small population to determine leucocyte profile in clinically graded three types of anemia as mild, moderate and severe of nutritional anemia

The determined hemoglobin percentage on comparison to control and within the grades of anemia class were statistically significant

There is significant increment in leucocyte count in anaemia in present study; this elevated leucocyte count (even within normal range) is associated with cardiovascular risk. Hypoxia induced by anaemia is a kind of stress, which increases vascular reactivity to catecholamine through glucocorticoids, thus helps in raising the leucocyte count.

So high leucocyte count might be seen as manifestation of a "hematological stress syndrome". Higher Leucocyte count carries a higher risk and seven fold more likely to develop cardiovascular vascular abnormality thrombotic phenomenon [12].

Our study showed that granulocyte count was significantly increased in nutritional anemia groups mainly constituted by increased in neutrophil counts which was statistically significant in severe anemia group of patients on contrary we noticed an statistically reduced count of eosinophil in all the groups of anemia class. As presented in various studies iron has important effects on both granulocyte functions and count. There are certain studies reporting increased basophil and neutrophil count in iron deficiency anemia patients Hrycek et al [13]). Regarding increased neutrophil count several studies have shown that iron deficiency induces changes in apoptotic response (Paino et al [14]), lower oxidative burst and oxidant product synthesis (Berrak et al [15]) resulting in increased neutrophils life span. Furthermore, iron deficiency anemia patients present reduced neutrophil phagocytic activity (Banerjee et al [16]). Thus the increased granulocyte cell count in iron deficiency anemia patients could compensates for the reduced phagocytic capacity.

Activated leucocytes particularly neutrophils release substances, i.e., neutral protease, cytotoxic material, hydrolytic enzymes that could lead to vascular and ischemic injuries, our results of decreased monocyte and eosinphils are in similar to the results obtained by K. Singh et al [12]. Exact reason of decreased count of these cells is not reported yet in anemic (ischaemic) conditions. Increased adhesiveness of these cells, i.e., monocyte, platelets, provoked by variety of stimuli may affect count of cells.

In our study, we found an increase in lymphocyte number distribution of subgroups in cases with iron deficiency anemia. While our study results are similar to the report stated by Canonne-Hergaux F et al [17] that lymphocyte proliferation response to mitogens remains normal in nutritional anaemia's in contrast to our study various reports are present which showed decrease in both lymphocyte number and lymphocyte blastogenesis and mitogenesis in iron deficiency anaemia [18].

Some results suggest lymphocyte dysfunction may be the result of functional defects of cells rather than quantitative defects. Recently neutrophil / lymphocyte ratio (NLR) has been described as significant inflammatory index in ischemic heart disease, myocardial infarction and even in different types of cancers i.e., colorectal. In multivariable models, after adjusting the chronic obstructive pulmonary disease, left ventricular ejection fraction, serum creatinine levels, long NLR, in addition to being an independent predictor and marker of mortality, out performs the prognostic information provided by elevated WBC count. In this way it explains the differential role played by neutrophils and lymphocytes in different conditions [12].

In view of above observation we attempted to study neutrophil / lymphocyte ratio in our observed samples and different grades of anemia, we noticed that their was decrease in NLR in all the grades of anemia on comparison to control group and the decrease in NLR was not statistically significant.
### Table 1: Basic characteristics of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>31.84 ± 5.14</td>
<td>31.46 ± 9.58</td>
<td>27.70 ± 5.17</td>
<td>31.48 ± 5.69</td>
<td>p&lt;0.14</td>
</tr>
<tr>
<td>Male (%) / Female (%)</td>
<td>25(50)/25(50)</td>
<td>22(44)/28(56)</td>
<td>25(50)/25(50)</td>
<td>27(54)/23(46)</td>
<td>p&lt;0.93</td>
</tr>
<tr>
<td>Ht (m)</td>
<td>1.53 ± 0.03</td>
<td>1.52 ± 0.02</td>
<td>1.53 ± 0.02</td>
<td>1.52 ± 0.02</td>
<td>p&lt;0.03</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>54 ± 6.06</td>
<td>53.46 ± 6.34</td>
<td>54.06 ± 3.90</td>
<td>50.72 ± 6.66</td>
<td>p&lt;0.19</td>
</tr>
<tr>
<td>BMI</td>
<td>22.73 ± 2.01</td>
<td>23.02 ± 2.41</td>
<td>23.12 ± 1.32</td>
<td>21.81 ± 2.54</td>
<td>p&lt;0.83</td>
</tr>
</tbody>
</table>

### Table 2: Comparison between anemic and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Anemia</th>
<th>Control</th>
<th>Mean difference</th>
<th>95% CI of difference</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>9.78±2.07</td>
<td>13.44±0.82</td>
<td>3.65</td>
<td>3.06-4.24</td>
<td>12.1</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>TLC</td>
<td>8070±2109</td>
<td>6860±1778</td>
<td>1210</td>
<td>555.5-1864.4</td>
<td>3.65</td>
<td>P&lt;0.0003</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>65.21±6.4</td>
<td>63.24±4.8</td>
<td>1.97</td>
<td>0.01-3.92</td>
<td>1.98</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>30.54±6.6</td>
<td>27.76±3.9</td>
<td>2.78</td>
<td>0.83-2.73</td>
<td>2.82</td>
<td>P&lt;0.005</td>
</tr>
<tr>
<td>Monocyte</td>
<td>2.14±1.4</td>
<td>4.68±1.9</td>
<td>2.54</td>
<td>2.04-3.04</td>
<td>10.11</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>2.12±1.3</td>
<td>4.32±2.2</td>
<td>2.21</td>
<td>1.69-2.74</td>
<td>8.35</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Basophil</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N/L ratio</td>
<td>2.28±0.75</td>
<td>2.35±0.55</td>
<td>0.06</td>
<td>-0.28-0.17</td>
<td>0.51</td>
<td>p&gt;0.05</td>
</tr>
</tbody>
</table>
Table 3: Variation in leucocyte profile in different grades of anaemia

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>f-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil</td>
<td>63.2±4.8</td>
<td>64.1±6.4</td>
<td>64.9±5.8</td>
<td>66.5 ±6.9 *</td>
<td>2.66</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>27.7±3.9</td>
<td>30.2±6.7</td>
<td>30.7±6.1 *</td>
<td>30.7 ±7.1 *</td>
<td>2.69</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Monocyte</td>
<td>4.7±1.9</td>
<td>2.6±1.4**</td>
<td>2.3±1.4**</td>
<td>1.5 ± 0.9 **</td>
<td>41.3</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>4.3±2.2</td>
<td>3.02±1.4**</td>
<td>2.02±1.4**</td>
<td>1.3± 0.5 **</td>
<td>38.17</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>N/L ratio</td>
<td>2.3±0.5</td>
<td>2.25± 0.7</td>
<td>2.26±0.7</td>
<td>2.3±0.5</td>
<td>0.23</td>
<td>p&gt;0.05</td>
</tr>
</tbody>
</table>

Figure 1: Comparison of haemoglobin concentration between control and anemia

Figure 2: Variation in leucocyte profile in different grades of anemia

REFERENCES:

