Study of some haematological and biochemical variables in children with iron-deficiency anaemia in Erbil-city

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Abstract
This study was carried out in pediatric out patients ward of Rapareen hospital in Erbil city, which included 45 children with iron deficiency anaemia (IDA), males and females, whose age range is between 1-5 years old, with 45 healthy children at same age and sex considered as control group, to determine the hematological and biochemical variables. The results indicate a significant decrease (p < 0.01) in haemoglobin, packed cell volume and red blood cell count in children with iron deficiency anaemia (IDA) when compared with control. White blood cell count, lymphocyte number, platelet count and red distribution width were increased significantly (p < 0.01) in children with IDA when compared with control. No significant differences observed in neutrophil number in children with IDA and control.

Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration decreased significantly (p < 0.01) in children with IDA when compared with control. Significant decrease (p < 0.01) noticed in serum iron and transferrin saturation in children with IDA when compared with control. While total iron binding capacity increased significantly (p < 0.01) in children with IDA when compared with control. Serum creatinine, urea, aspartate transaminase and alanine transaminase activity did not show significant differences in children with IDA and control group.

درسة بعض المتغيرات الدموية و الكيميائية عند الأطفال المصابين بفقر الدم نقص الحديد
في مدينة أربيل

كتنو عفاش مولود

المستخلص
تم إجراء هذا البحث في وحدة الأطفال في مستشفى رابرين في مدينة أربيل والذي شمل 45 طفل (ذكور و إناث) عمرهم 1-5 سنوات يعانون من فقر الدم نتيجة نقص الحديد و 45 طفل سليم بنفس العمر والجنس واعتبر كمجموعة تحكم لدراسة التغيرات التي تطرأ في بعض العوامل الدموية و الكيميائية. أظهرت النتائج انخفاض معنوي (p < 0.01) في متوسط خصائص الدم، هemmoglobin،packed cell volume و red blood cell count في الأطفال الذين يعانون من فقر الدم نقص الحديد مقارنة مع مجموعة التحكم. حيث ازدادت في كبار الداء البيض، معدل التوزيع الحيوي، معدل العدد المقابل للمجموعة (p < 0.01) في الأطفال الذين يعانون من فقر الدم نقص الحديد. ولم يتم رصد أي اختلاف معنوي في عدد الخلايا العقلية في الأطفال الذين يعانون من فقر الدم نقص الحديد مقارنة بمجموعة التحكم. كما ارتبط مستوى الكرياتينين في الدم ومستوى الكرياتينين في المصل ومستوى الفينول打着 معنوي (p < 0.01) في الأطفال الذين يعانون من فقر الدم نقص الحديد مثلاً مقارنة بمجموعة التحكم. بينما لم يتم رصد أي اختلاف معنوي في الأطفال الذين يعانون من فقر الدم نقص الحديد مقارنة بمجموعة التحكم. على ترتيب ترتيب ترتيب أ، ب، حيث تم رصد اختلاف معنوي (p < 0.01) في الأطفال الذين يعانون من فقر الدم نقص الحديد مثلاً مقارنة بمجموعة التحكم.
Introduction
The prevalence of nutritional iron deficiency anaemia in infants and toddlers has declined dramatically since 1960. However, satisfaction with this achievement must be tempered because iron deficiency anaemia in infants and toddlers is associated with long lasting diminished mental, motor, and behavioral functioning (1). Iron deficiency develops in three stages. Firstly, there is a reduction in serum ferritin which is directly related to iron reserves. Secondly, a reduction in serum iron concentration and an increase in iron binding capacity, the third stage occurs with restriction in the synthesis of haemoglobin, which may lead to anaemia. In iron deficiency anaemia (IDA), red cells undergo morphological change from normocytic and normochromic to microcytic and hypochromic (2, 3). Iron deficiency anaemia is characterized by a defect in haemoglobin synthesis, resulting in red blood cells that are abnormally small (microcytic) and contain a decreased amount of haemoglobin (hypochromic) (4). Iron is essential at all cells functions of iron include involvement in energy metabolism, gene regulation, cell growth and differentiation, oxygen binding and transport, muscle oxygen use and storage, enzyme reactions, neurotransmitter synthesis, and protein synthesis (5). The World Health Organization and Center for Disease Control and Prevention, haemoglobin cut-points for diagnosing anaemia have been widely adopted: in infancy and childhood < 11g/dl for 0.5 - 4.9 years (6). Haemoglobin concentration and haematocrit are frequently used to screen for iron deficiency because of their low cost and wide standard availability. However, haemoglobin concentration and haematocrit are only decreased in full-blown IDA, not in iron deficiency, reduced hemoglobin concentration and hematocrit can be found in many other conditions, such as folate deficiency, Vit. B12 deficiency, thalassemia, sickle cell disease, anemia of chronic disease and chronic renal failure (7, 8). Transferrin is a major serum iron transport protein and is usually increased in iron deficiency and decreased in iron overload, transferrin saturation % is not considered a consistently reliable indicator of iron deficiency (9), in a study blood was obtained from 52 children 12-35 months old, the positive predictive value of Hb concentration < 110 g/L for ID was 29% and the sensitivity was 30% (10). Allen with coworker (2000) observed lower haematocrit alteration in anaemia children. IDA is a significant risk factor for stroke in otherwise healthy young children and had lower median hemoglobin level and MCV and a higher median platelet count in children 1-3 years (12). Haemoglobin concentration decreased to < 110g/L and mean corpuscular volume was less than 70 fl in IDA children aged 6-36 months (13). Significant lower of mean corpuscular volume and mean corpuscular haemoglobin while was found in anaemic children (14). Iron deficiency is accompanied by reductions in serum iron and transferrin saturation and by elevation in red cell distribution width and total iron binding capacity (15). The total leucocyte count and lymphocyte counts were increased in 40 children had iron deficiency anaemia (16). The results of (17) suggest that children with IDA have impaired renal tubular function, while (18) demonstrated that the serum creatinine and urea were within normal range in iron deficiency anaemia children. It has also been recognized that serum creatinine and urea levels were similar in IDA group and control (19). Serum creatinine
level in anaemic children (1-15 years old) suffering from leukemia or solid tumor is normal and not different from those in the control group (20). Aspartate transaminase was normal in children with IDA before iron treatment but increased markedly with iron supplementation (21). The aim of this study was to determine and demonstrates the association between haemoglobin, haematocrit, erythrocyte count, serum iron and total iron binding capacity in children with IDA in Erbil city.

Patients and methods
This study was carried out in the pediatric out patients ward of Rapareen hospital in Erbil city for establishing the haematological and biochemical variables of iron deficiency anaemia in children between 1-5 year of age from 19-6-2008 to 23-12-2008. A total of 45 pre school children (includes 25 males and 20 females) were diagnosed to have iron deficiency anaemia (Hb level < 11 g/dL) and blood film was prepared for each child and the definit diagnosis of IDA is when transferrin saturation % is below 15%. All children had normal growth and normal serum total protein, the only etiology of iron deficiency anemia in all children is nutritional iron deficiency, personal and family data were obtained from each children on a single occasion by interviewing the parents or responsible person. The control group consists of 45 children at the same age and sex of children with IDA. Vein puncture blood (6ml) 2 ml of it was collected into tubes containing EDTA for analysis of haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cell count (RBC), White Blood Cell count (WBC), platelet count, Neutrophil number and Lymphocyte number, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red distribution width (RDW) by using a coulter counter (model cell counter, No. 220-240 mode cell-Tech. EU) other 4 ml of blood was promptly separated from clot after centrifugation for 10 min at 3000 rpm, serum iron (SFe), total iron binding capacity (TIBC), creatinine, urea, aspartate transaminase (AST) and alanin transaminase (ALT) were determined by colorimetric method by using kit (22,23,24,25). And % transferrin saturation was calculated from serum iron and TIBC. Statistical analysis was done by using t-test paired equal variance. A P < 0.01 value was considered statistically significant. All value is presented as mean ± SE (26).

Results
There were no statistically significant differences in age p=0.37 and sex p=0.61 in children with iron deficiency anemia (IDA) and control group age p=0.71 and sex p=0.46. Significant differences were observed in haematological and biochemical variables in children with IDA and control group. Table (1) demonstrate a significant decrease (p < 0.01) in haemoglobin (Hb), packed cell volume (PCV) and red blood cell count (RBC) in children with IDA when compared with control. While white blood cell counts (WBC), lymphocyte number, platelet count and red distribution width (RDW) increased significantly (p < 0.01) in children with IDA when compared with control. Neutrophil number in children with IDA decreased non significantly (p > 0.01) when compared with control. According to RBC indices the data in this study show that the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) decreased
significantly (p < 0.01) in children with IDA when compared with control. Significant decrease (p < 0.01) was shown in level of serum iron and transferrin saturation (TS) in children with IDA when compared with control, and significant increase (p < 0.01) in level of total iron binding capacity in children with IDA when compared with control (Table 2). The data in Table (3) shows no significant differences (p > 0.01) in level of serum creatinine, urea, aspartate transaminase (AST) and alanine transaminase (ALT) activity in children with IDA and control. Table (4) observed the best correlation between haemoglobin and packed cell volume (r = 0.7193) and RBC count (r = 0.5378) and MCV (r = 0.9940). Packed cell volume showed correlation with RBC count (r = 0.7450). Negative correlation was found between RBC count and MCV (r = -0.4756), and MCH (r = -0.4824), and negative correlation between platelet count and MCV (r = -0.4914) and MCH (r = -0.4257) and between RDW with MCV (r = -0.4633) and MCH (r = -0.4935), and MCHC (r = -0.5791) and correlation between RDW with TS (r = 0.4537) was shown, higher correlation between MCV and MCH (r = 0.9620) and MCHC (r = 0.5664), and correlation between MCH and MCHC (r = 0.6661) with negative correlation between MCH and TIBC (r = -0.3784) was observed.
Table (1): Mean ± SE of hematological variables in children with IDA and control group.

<table>
<thead>
<tr>
<th></th>
<th>Hb</th>
<th>PCV</th>
<th>RBC</th>
<th>WBC</th>
<th>Lymphocyte</th>
<th>Neutrophil</th>
<th>Platelet</th>
<th>RDW</th>
<th>MCV</th>
<th>MCH</th>
<th>MCHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>10.1±0.16</td>
<td>32.22±0.12</td>
<td>4.06±0.018</td>
<td>10.40±0.87</td>
<td>32±12.95±35.33</td>
<td>431±11.73±36.42</td>
<td>368.8±3.62</td>
<td>15.58±0.057</td>
<td>75.04±1.40</td>
<td>22.48±0.46</td>
<td>30.44±0.18</td>
</tr>
<tr>
<td>Control</td>
<td>12.76±0.026</td>
<td>40.04±0.076</td>
<td>4.74±0.097</td>
<td>8.59±0.045</td>
<td>1580±13±8.03</td>
<td>4876±62±9.22</td>
<td>294.86±1.98</td>
<td>13.66±0.15</td>
<td>87.13±1.06</td>
<td>28.08±0.055</td>
<td>31.40±0.023</td>
</tr>
<tr>
<td>t-test (0.01)</td>
<td>11.053**</td>
<td>7.934**</td>
<td>4.848**</td>
<td>2.49</td>
<td>6.717**</td>
<td>2.241</td>
<td>2.667*</td>
<td>4.656**</td>
<td>6.882**</td>
<td>9.481**</td>
<td>3.991**</td>
</tr>
</tbody>
</table>

* = significant, ** = higher significant, without star = no significant.

Table (2): Mean ± SE of serum iron, Total iron binding capacity and Transferrin saturation in children with IDA and control group.

<table>
<thead>
<tr>
<th></th>
<th>Iron µg/dL</th>
<th>TIBC µg/dL</th>
<th>TS %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>28.66±0.074</td>
<td>520.35±0.67</td>
<td>15.26±0.066</td>
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<tr>
<td>Control</td>
<td>72.82±0.44</td>
<td>367.28±1.18</td>
<td>37.84±0.15</td>
</tr>
<tr>
<td>t-test (0.01)</td>
<td>14.629**</td>
<td>16.729**</td>
<td>19.931**</td>
</tr>
</tbody>
</table>

** = higher significant

Table (3): Mean ± SE of Serum creatinine, Urea, AST and ALT in children with IDA and control group.

<table>
<thead>
<tr>
<th></th>
<th>Creatinine mg/dL</th>
<th>Urea mg/dL</th>
<th>AST IU/L</th>
<th>ALT IU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>0.60±0.044</td>
<td>14.37±0.064</td>
<td>17.46±0.18</td>
<td>20.2±0.95</td>
</tr>
<tr>
<td>Control</td>
<td>0.57±0.049</td>
<td>15.82±0.065</td>
<td>15.22±1.10</td>
<td>18.6±0.20</td>
</tr>
<tr>
<td>t-test (0.01)</td>
<td>0.746</td>
<td>2.351</td>
<td>1.354</td>
<td>0.958</td>
</tr>
<tr>
<td></td>
<td>0.0124</td>
<td>0.0234</td>
<td>0.0345</td>
<td>0.0456</td>
</tr>
<tr>
<td>----</td>
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<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>1</td>
<td>0.0098</td>
<td>0.0109</td>
<td>0.0120</td>
<td>0.0131</td>
</tr>
<tr>
<td>2</td>
<td>0.0087</td>
<td>0.0098</td>
<td>0.0109</td>
<td>0.0120</td>
</tr>
<tr>
<td>3</td>
<td>0.0077</td>
<td>0.0087</td>
<td>0.0097</td>
<td>0.0107</td>
</tr>
<tr>
<td>4</td>
<td>0.0067</td>
<td>0.0077</td>
<td>0.0087</td>
<td>0.0097</td>
</tr>
</tbody>
</table>

**Table (4): Correlation (values of r) between hemato logical and biochemical variables of children with IDA.**

DL = 0.01, T = 0.376, Level of significance = 0.01, * = significant.
Discussion

Several studies have shown that iron deficiency anaemia is particularly prevalent in infants whom there has been a pucity of information regarding dietary intake, particularly iron intake (27, 28). 45 children with iron deficiency anaemia participated in this study; a decreased concentration of haemoglobin was reported. Hypochromic microcytic anaemia is frequently encountered in clinical practice, in most cases they are due to iron deficiency results in a decrease production of haemoglobin (29). A haemoglobin value of 11g/dl was used as a cut-off point for the diagnosis of anaemia in children at the age of nine month (30). Significant decrease in PCV and RBC count were observed in the present study which agree with the study of Thurlow with colleague (2005) which demonstrate that the children with IDA had lower PCV with MCV which associated with haemoglobinopathies and thalassemias as well as with deficiencies of iron and vitamine A. Result of pre school children in Haiti observed a correlation between Hb and age and suggested that children are able to consume a more varied diet with increasing age, thus improving their hematological indicators (32), while disagree with King with colleague (2005) which showed significant increase in RBC count in children with IDA due to decreased haemolysis and increased red cell survival in the IDA group. WBC count, lymphocyte number, platelet count and RDW were increased significantly in children with IDA, this result is in agreement with Kuvibidila with colleague (1999) which show that the protein kinase C activity and translocation of both splenic and purified T cells were altered by IDA, and Walter with coworker (1986) suggest that iron does not have a direct effect on circulation neutrophils but, rather, that it is required during the development of neutrophils in the bone marrow. RDW seems to be the first haematological manifestation of iron, and is more sensitive screening for IDA than serum iron or serum ferritin, also allowing the differential diagnosis of iron deficiency anaemia and thalassemia (35). MCV, MCH and MCHC decreased significantly in children with IDA in this study, similar results suggested by Hadler with coworker (2002) in infants aged between six to twelve months which found grater correlation between haemoglobin and MCV, MCV and MCH. According to platelet count the Gupta (2001) observed that the iron is required for the synthesis or production of platelets. Thus, when iron deprivation occurs, it first affects inhibitor compartment, leading to thrombocytosis; once the iron deficiency is sever enough to deplete the iron in the essential component compartment, thrombocytopenia occurs. Serum iron with % TS decreased significantly and this result agree with Eden and Mir (1997) which suggest that the children at 1-3 year old have the lowers daily iron intake of any age group across the lifespan, at one year, breast feeding or iron-fortified milk formula is often replaced with cow's milk ,non iron-fortified cereals enter the diet, and Satowitz and Oskie (1983) which reported that the infants, the introduction of cow's milk in the first year of life is the greatest dietary risk factor for the development of iron deficiency anemia, while Dallman with coworker (1080) showed that the cow's milk is low in iron, and its iron is poorly absorbed and decreases the absorption of iron from other dietary sources. Significant increase in TIBC was observed and this result is disagree with Agarwal with coworker (1981) which reported that the various factors
that reduce the transferrin level, infections, inflammation and proteinuric conditions and hence under nutrition appeared to be the chief cause of lowered TIBC.

References
Pediatric Nephrol., 18: 254-256.