Research Article

Evaluation of Urinary Kidney Injury Molecules in Children with Iron-Deficiency Anemia

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Abstract

Introduction: Anemia is characterized by a decrease in the amount of erythrocytes or hemoglobin concentration in the blood. **Aim of the work:** This study was aimed to evaluate whether patients with IDA have subclinical renal injury which can be determined by urinary kidney injury marker KIM-1.
Patients and Methods: This study included 60 children, 29 with iron deficiency anaemia and 30 apparently healthy children serving as controls matched in age and sex. Iron deficiency anaemia children were selected from the General Clinic at El Minia University Hospital during the period from January 2016 to june 2016. **Results:** This study was done over the period from Jan to June 2016 at EL-Minia University Hospital Department of Pediatrics. **Discussion:** Anemia is a common disease in children. Although there are various types of anemia, iron-deficiency is the most common cause of anemia in childhood. Iron-deficiency anemia can affect functions of many organ systems, especially neurocognitive functions. One of the functions of iron in the human body is to deliver oxygen throughout the body by using the structure of hemoglobin. **Recommendation:** From our study we recommend that: The results of this study suggest that patients with IDA may have renal injury determined by urinary kidney injury markers, including KIM-1. Reduced oxygen delivery to kidneys and altered cell function due to anemic hypoxia may be key factors. Further studies are needed to determine the mechanism of kidney injury in IDA Follow up for after management of iron deficiency. We have to exclude ankylstoma infection.
Keywords: Cr: Creatinine, DCYTB: Duodenal Cytochrome B, DMT1: Divalent Metal Ion Transporter 1

Introduction

Anemia is characterized by a decrease in the amount of erythrocytes or hemoglobin concentration in the blood (Charles et al., 2012). Anemia is seen in all age groups . According to the World Health Organization, 1.62 billion people are affected by anemia (Charles et al., 2012). Iron-deficiency anemia (IDA) is responsible for half of all anemia cases. It has been stated that 273,000 individuals die due to iron-deficiency anemia yearly (Pasricha et al., 2013).

Iron is transported in the blood by a delivery protein called transferrin and is found in hemoglobin at the highest concentration. Hemoglobin is a protein that delivers oxygen into the tissues of the body. Failure occurs in hemoglobin production and oxygen delivery to tissues in individuals with iron deficiency, causing tissue hypoxia to develop (Nangaku, 2006).

Kidney injury is one of the major causes of mortality and morbidity (Argyri et al., 2013). Hypoxia plays a crucial role in the pathogenesis of kidney injury . In previous studies (Du et al., 2014). It has been reported that anemia causes tissue hypoxia in kidneys earlier and at a higher hemoglobin concentration than in other organs (Ragoonanan et al., 2009). Clinically, serum creatinine and blood urea levels are commonly used to diagnose kidney injury. However, a disadvantage is that these parameters are affected by age, sex, muscle mass, dehydration, and drugs. In addition, creatinine level does not increase unless at least half of the renal function is lost (Martensson et al., 2012). Therefore, new markers are needed to diagnose kidney injury. It has been shown that human kidney injury molecule (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-b-Dglucosaminidase (NAG), and liver-type fatty acid-binding protein (L-FABP) have advantages

in diagnosing kidney injury (McCullough et al., 2013).

Iron-deficiency anemia is a common type of anemia during childhood. In a search of the literature we found few studies on the effect of iron-deficiency anemia on kidney function using kidney injury molecules. Thus, the aim of the present study was to determine urine kidney injury molecule levels in order to evaluate subclinical kidney injury in pediatric patients with IDA(Scott et al.,2014).

Aim of the work

This study was aimed to evaluate whether patients with IDA have subclinical renal injury which can be determined by urinary kidney injury marker KIM-1.

Patients and Methods

This study included 60 children, 29 with iron deficiency anaemia and 30 apparently healthy children serving as controls matched in age and sex. Iron deficiency anaemia children were selected from the General Clinic at El Minia University Hospital during the period from January 2016 to June 2016.

The children divided into 2 groups:

1- Group 1 (cases) consists of 29 children with iron deficiency anaemia. Their age ranges from 4 -11 years and contains 16 males (55.2%) and 13 females (44.8%).

2- Group 2 (controls) consists of 30 children healthy control. Their age ranges from 3.5 -11.7 years and contains 16 males(53.3%) and 14 females (46.7%).

Inclusion criteria:

1- Patients diagnosed as nutritional iron deficiency anaemia according to (history, clinical examination and laboratory investigations).

2- Age: from 6 monthes-17years.

Exclusions Criteria:

1-patients taking any drug that potentially affects kidney function, such as non-steroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, or diuretics.

2-Children having a disease such as diabetes, rheumatic disease, urinary tract infection, sepsis, hypoxia, or diseases of the liver, kidneys, heart, or lungs.

In addition, thalassemia patients were excluded by performing hemoglobin electrophoresis.

All cases were subjected to the following after explaining the study to parents and informed consent was obtained;

1. Full Medical History including:

a) Birth history, vaccination, detailed nutritional history, past medical and surgical histories including history of recurrent illnesses, developmental history, drug history, history of allergy and family history.

2. Clinical Assessment:

a) A complete systemic examination, lay a concentration on sign of anemia.

b) Auxological assessment of growth parameters including (weight in Kg, height in Cm & BMI) using Growth Charts **(**Flegal et al., 2001).

3. Investigations: U all done in one visit.

a) Laboratory:

- CBC (Hb, RBCs, Hct, MCV, MCH, MCHC).
- S. Iron, S. Ferritin and TIBC (Total Iron Binding Capacity).
- Renal function tests was done for all patients using fully automated clinical chemistry auto analyzer system Konelab 20i (Thermo Electron Incorporation, Finland).

The laboratory machines used are:

- SYSMEX XT-1800i and SYSME XS-500i; for CBC.
- INTERGRA 800; for S. Iron and TIBC.
- COBAS e 601; for Ferritin.
- Human Kidney Injury Molecule 1 (Kim-1) ELISA Kit

The normal level of Iron ranges between (6.6-26 umol/l), Ferritin (15-150 ng/ml), TIBC (41-77 umol/l) and MCV (79.4-94.8 fl).

After receiving informed consent from the patients and their caregivers, 5 ml urine and 7ml blood samples were obtained. Anemia in childhood was defined as a hemoglobin (Hb) concentration below the cut-off levels established by the World Health Organization:

<11 g/dl in children aged 6–59 months, <11.5 g/dl in children aged 5–11 years, and 12 g/dl in older children (aged 12–17).

IDA was diagnosed when the serum iron level was <60 microgram/dL (N=60–150 micro-

gram/dL), iron-binding capacity (TIBC) over 360 μg/dL, transferrin saturation <15% (normal 20–50), and decreased serum ferritin <20 μg/L (normal 40–200 μg/L).

Transferrin saturation was calculated as ×100 serum iron/iron-binding capacity. After obtaining the necessary blood samples, iron therapy was initiated in all patients.

The urine samples were tested for urinary tract infection. The urine samples, collected from the patients who did not have a urinary tract infection, were centrifuged for 3 minutes at 3000 RPM. Centrifuged urine was placed into Eppendorf tubes and stored at −80°C.

Afterwards, urine was measured for KIM-1 level. This variable was measured by enzyme-linked immunosorbent assay (ELISA) method according to the manufacturer's instructions. Its detection range in our kit is 0.312 ng/ml-20 ng/ml

Serum creatinine values were evaluated according to age and sex.

Human Kidney Injury Molecule 1 (Kim-1) ELISA Kit

For the quantitative determination of human kidney injury molecule 1 (Kim1) concentrations urine.

Principle Of The Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for Kim-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Kim-1 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for Kim-1 is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Kim-1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Results

This study was done over the period from Jan to June 2016 at EL-Minia University Hospital Department of Pediatrics.

This study included:

1- Group 1 (cases) consists of 29 children with iron deficiency anaemia. Their age ranges from 4 -11 years and contains 16 males (55.2%) and 13 females (44.8%).

2- Group 2 (controls) consists of 30 children healthy control. Their age ranges from 3.5 -11.7 years and contains 16 males (53.3%) and 14 females (46.7%).

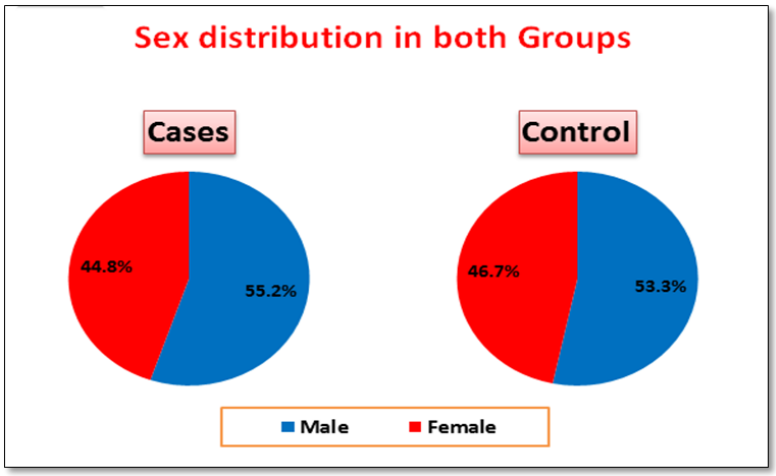
Table 1: Baseline characteristics of the study population.

	Cases (n=29)	Control (n=30)	P value
Age			
Range	(4-11)	(3.5-11.7)	0.614
Mean ± SD	7.05±1.81	7.33±2.31	
Sex			
Male	16(55.2%)	16(53.3%)	0.887
Female	13(44.8%)	14(46.7%)	

Independent sample t test for parametric quantitative data between the two groups
Chi square test for qualitative data between the two groups
***: significant difference at p value < 0.05**

The previous table showed that this study included 29 children (16 males and 13 females) diagnosed with iron-deficiency anemia (IDA), and 30 healthy children (16 males and 14 females) in the control group. The mean age of patients was 7.05±1.81 years, and that of the control group was 7.33±2.31 years. There were no significant differences in mean age or sex distribution between IDA and control groups (p=0.614; p=0.887, respectively). (Table 1).

Figure: Sex distribution in both groups.



The previous table showed no significant differences in mean sex distribution between IDA and control groups.

Discussion

Anemia is a common disease in children. Although there are various types of anemia, iron-deficiency is the most common cause of anemia in childhood. Iron-deficiency anemia can affect functions of many organ systems, especially neurocognitive functions. One of the functions of iron in the human body is to deliver oxygen throughout the body by using the structure of hemoglobin (Subramaniam and Girish, 2015).

Iron is transported through the protein transferrin in the blood and is found mostly in the form of hemoglobin. The most important function is iron binding oxygen reversibly on the center of "heme", a protein transferring oxygen in the body. Four iron ions are needed for each hemoglobin unit, located in the structure of each unit of hemoglobin heme binds as a tetramer. This last phase in the formation of hemoglobin cannot occur in the case of iron deficiency and not enough heme can be produced (Fufaa et al., 2015).

When there is heme deficiency, globin biosynthesis is automatically suppressed, along with the effectiveness of heme-regulated transcriptional inhibitor (HRI). HRI activity, increasing as a direct result of heme deficiency, suppresses the synthesis of the globin.
In addition, it leads to suppression of the heme transcription initiator, which is a key factor in the synthesis. Ultimately, hemoglobin cannot be formed and anemia develops as a consequence of the suppression of the heme with globin synthesis (Fufaa et al., 2015).

Oxygen delivery to the tissue is affected by the Hb concentration in the blood. The decrease in Hb reduces the tissue oxygenation and leads to hypoxia. Hypoxia affects the brain, liver, and, especially, the heart. Since the kidneys are very sensitive to hypoxia, low oxygen pressure in the blood plays an important role in the pathophysiology of kidney injury. Although anemia causes the destruction of tubule-interstitial cells by stimulating hypoxia and leading to acute kidney injury (Schreiber, 2006), the effect of IDA on the kidneys may be due to chronic hypoxia (Mohanram et al., 2004).

Recommendation

From our study we recommend that:
The results of this study suggest that patients with IDA may have renal injury determined by urinary kidney injury markers, including KIM-1
Reduced oxygen delivery to kidneys and altered cell function due to anemic hypoxia may be key factors.
Further studies are needed to determine the mechanism of kidney injury in IDA
Follow up for after management of iron deficiency.
We have to exclude ankylstoma infection.

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