J. Basic. Appl. Sci. Res., 1(10)1406-1410, 2011

ISSN 2090-424X
Journal of Basic and Applied Scientific Research
www.textroad.com

Renal Tubular Function in Saudi Children with Iron Deficiency Anemia

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ABSTRACT

Iron deficiency anemia (IDA) resulting from lack of sufficient iron for synthesis of haemoglobin is the most common hematological disease of infancy and childhood. Researchers have investigated the deleterious effects of IDA on the cardiovascular and nervous systems, but the impact of this condition on renal function has not been examined in depth. The aim of the present study was to examine the renal tubular function in children with iron deficiency anemia. A case control study was performed including 100 children with iron deficiency anemia and 50 healthy age matched control. Blood and urine samples were obtained for haematological and biochemical investigation. Statistical analysis was performed by unpaired sample *t* test, and linear regression. We found that the mean urine β2 microglobulin level and mean urinary N-acetyl-B-D-glucosaminidase/creatinine (UNAG/CR) were significantly higher in the children with iron deficiency anemia than in controls ($P<0.05$). There was significant negative correlation between Hb levels and UNAG/Cr and urine β2 microglobulin in the group of children with IDA anaemia ($r= -0.77$ $P<0.001$, $r= -0.64$, $P<0.001$), respectively. We conclude that the urine β2 microglobulin and UNAG/CR were higher in children with iron deficiency anemia than in normal subjects. These findings provide evidence of renal tubular dysfunction in IDA.

KEY WORDS: Iron deficiency anemia, Renal tubular function, Microglobulin. N-Acetyl-β-D-glucosaminidase.

INTRODUCTION

Anemia is a common problem in childhood, especially in children aged six to 24 months old. It has been estimated that among children below four years of age, 12% are anaemic in developed countries and 51% are anaemic in developing countries [1]. Iron deficiency anemia (IDA) is the most commonly recognised form of nutritional efficiency in developing [2] and developed countries [3]. About 600 million individuals worldwide have IDA [1] which in most cases, is of dietary origin, such as inadequate weaning [3]. IDA can no longer be considered a simple anemia that can be readily reversed by iron therapy. Iron deficiency (ID) may be associated with abnormalities in cell-mediated immunity and the ability of neutrophils to kill several types of bacteria [4], and poorer psychomotor development and behavioural changes of young children [5]. Children who have IDA in infancy are at risk for long-lasting developmental disadvantages [6], as well as behavioural and developmental disturbances [7], compared with their peers with a better iron status. Iron is necessary for maintaining normal structure and functions of virtually all mammalian cells, and is also involved in the immune and non-immune host defence. *In vitro* studies have shown that iron and iron-binding proteins are important for lymphocyte proliferation, satisfactory functioning of natural killer cells, B cells and antibody production and the activity of phagocytic cells [8]. Thus, it has been suggested that infants and toddlers should be screened for ID. Screening for anemia, conducted mostly in Western countries, has detected a higher prevalence of ID among Asian children [9, 10].

Urinary N-Acetyl-β-D-glucosaminidase (NAG) is a high molecular weight lysosomal enzyme that shows high activity in renal proximal tubular cells. It is usually not filtered in the glomerulus and its increased levels in urine reflect proximal tubular dysfunction of the kidney. NAG remains stable in urine and can be determined easily [11].

The possible impact of IDA on renal function has not been investigated widely. Since renal ischemia is characterized predominantly by tubular damage, we hypothesized that children with IDA might develop tubular dysfunction due to chronic renal hypoxia. Hypothetically, disturbed mitochondrial energy metabolism in IDA might also cause renal tubular cell dysfunction. Therefore we designed a study to assess renal tubular function in children with IDA.

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METHODS

Patients and Methods

A case control study was carried out on children aged 6-36 months who attended the paediatrics out patient clinics; Ohoud hospital Al-Madinah Al-Munawarah during the period from September 2009 to August 2010. The study population included 100 children with IDA, and 50 healthy age and sex matched randomly selected children attended the well baby clinic for the follow-up or vaccination as a control group.

All the included children were subjected to haemoglobin evaluation to confirm their anaemic status. The children were termed anaemic keeping the WHO's criteria stated that haemoglobin (Hb) <11g/dl for children aging 6 months to 6 year. Children who were already diagnosed as anaemic and were on haematinics and /or complaining of urinary tract infection, malnutrition, renal disease, glucose-6-phosphate dehydrogenase (G6PD) deficiency and hemoglobinopathy were excluded depending on the clinical and the laboratory criteria.

Biochemical Parameters

A blood sample was collected from each subject to determine haemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), and red blood cell distribution width (RDW). Healthy children with normal Hb, Hct, MCV, and RDW served as controls. If Hb, Hct, MCV, and RDW suggested IDA (Hb<11 g/dl, Hct<32%, MCV<72 fl, RDW>15%), a second blood sample was drawn to determine the levels of serum iron, iron-binding capacity and ferritin. Transferrin saturation was also calculated from the formula of serum iron level/iron-binding capacity level x 100. Criteria for iron deficiency anemia were: serum iron level<50 μg/dl, iron binding capacity >352 μg/dl, ferritin level <12 ng/ml, and transferrin saturation <15%. Serum sodium and creatinine were also determined in all subjects, including controls. Fractional excretion of sodium (FENa %) was calculated from the formula:-

FENa %= urinary Na / serum Na x 100 / urine creatinine / serum creatinine [12]. Fresh second-morning urine samples were obtained from subjects in both groups. Immediately after collection, an aliquot from each sample was frozen for enzyme analysis, and the remaining urine was cultured, microscopically examined and tested for osmolality and levels of protein, glucose, microalbumin, β2 microglobulin, creatinine, sodium and calcium. Patients with urinary infection or malnutrition were excluded. Urinary N-acetyl-β-D-glucosaminidase (NAG) was measured in the frozen sample using the colorimetric method. [13]. In the current study since the range of age was narrow, creatinine-corrected values were used. Moriguchi et al [14] recommended the use of the uncorrected, observed values of the markers rather than the traditional creatinine-corrected values when comparison covers people of a wide range of ages.

Ethical Consideration

The study was approved by the Ethics and Human Research Committees of the Ohoud Hospital, Al-Madinah Al-Munawwarah, Kingdom of Saudi Arabia. Verbal consent was obtained from parents of all children. A brief counselling regarding iron deficiency anemia together with clarification of the aim and method (regarding blood sampling) were given.

Statistical Analysis

Statistical evaluation of all data was conducted using the Statistical Package for Social Studies version 13.0 (SPSS, Chicago, IL, USA). The Quantitative data were presented as mean ± standard deviation (SD). Statistical analysis was carried out using chi-square test, non parametric Mann Whitney U-test and the linear regression analysis. All tests were two tailed and considered significant when $p<0.05$ and highly significant when $p<0.001$.

RESULTS

The demographic parameters, haematological and biochemical parameters of IDA cases and healthy subjects are shown in (Table 1).

In the IDA patient group, the mean Hb level was 8.6 ± 0.7g/dl, 35% of patients had Hb level less than 8 g/dl, Hct, MCV and RDW values were 28.8 ± 2.6%, 64±5.4 fl, and 18.4 ± 1.9%, respectively. The corresponding findings for the control group were 12.9 ± 0.5 g/dl, 36.8±1.6%, 77.7 ± 4 fl, and 13.5 ± 0.8%, respectively. For all these parameters, the patient and control results were significantly different ($P <0.001$ for all). In the IDA patient group, the mean levels of iron, iron-binding capacity, and ferritin were 34 ± 9 mg/dl, 362 ± 60 mg/dl, and 11.9 ± 4.9 ng/ml. There were no differences between the patient and control means for serum sodium (137.5 ± 3 mEq/l versus 137.6 ± 3.9 mEq/l, respectively), serum creatinine (0.48 ± 0.5 mg/dl versus 0.48 ± 0.1 mg/dl), or urine osmolality (588 ± 201 mosmol/kg versus 591 ± 200 mosmol/Kg).

The results of the IDA group for urinary microalbumin and calcium/creatinine ratio (Ca: Cr) were 0.82 ± 0.85 mg/dl and 0.22 ± 0.8, respectively, and the corresponding control findings were 1 ± 0.8 mg/dl and 0.20 ± 0.2 ($P> 0.05$ for both comparisons). Urine β2 microglobulin level was increased significantly ($P <0.05$) in IDA cases (1.6 ± 0.80) than in controls (0.10 ± 0.15). There was a negative correlation between Hb level and urine β2 microglobulin level ($r = -0.81$, $p< 0.001$). The FENa was not significantly higher in the IDA group than in the

controls [0.75±0.28% versus 0.63±0.3 %, respectively (P <0.05). Also, urinary NAG/creatinine [UNAG/Cr (U/mg)] was significantly higher in patients with IDA than in controls [25.2±30 versus 15.90±11.9, respectively (P <0.001).

In IDA patients, there was a negative correlation between Urine β2 microglobulin and serum ferritin and serum iron (r =-0.71, P <0.001) and (r=0.65, P <0.001) respectively. UNAG/Cr was negatively correlated to both serum ferritin and serum iron (r = -0.66, P <0.001, r = -0.70, P<0.001), respectively. Our results showed that using linear regression there was significant negative relation between Hb levels and UNAG/Cr and Urine β2 microglobulin in the group of children with IDA anaemia (r = -0.77 P <0.001, r = -0.64 , P <0.001), respectively. There was a positive correlation between UNAG/Cr and Urine β2 microglobulin (r =0.89, P <0.001).

Table 1: Summary of haematological and biochemical data of patients with iron deficiency anaemia (IDA) and controls.

	IDA patients n=100	Controls n=50	P- value
Female/male	58/42	26/24	P>0.05 ^{NS}
Age (months)	11±9	10±8	P>0.05 ^{NS}
Hb (g/dl)	8.6±0.1	12.9±0.5	P<0.001**
Hct (%)	28.8±2.6	36.8±1.6	P<0.001**
MCV (fl)	64±5.4	77.7±4.0	P<0.001**
RDW (%)	18.4±1.9	13.5±0.8	P<0.001**
Serum iron (mg/dl)	34±9	------	------
Serum iron-binding capacity (mg/dl)	362±60	------	------
Serum ferritin (ng/ml)	11.9 ±4.9	------	------
Serum sodium (mEq/l)	137.5±3	137.6±3.9	P>0.05 ^{NS}
Serum creatinine (mg/dl)	0.48±0.5	0.49±0.1	P>0.05 ^{NS}
Urine osmolality (mosmol/kg)	588±201	591±200	P>0.05 ^{NS}
Urine microalbumin (mg/dl)	0.82±0.75	1.0±0.8	P>0.05 ^{NS}
β2 microglobulin (µg/dl)	1.6± 0.80	0.10± 0.15	P<0.001**
Ca/Cr (mg/mg)	0.22±0.8	0.20±0.2	P>0.05^{NS}
FENa (%)	0.75±0.28	0.63±0.3	P<0.05*
UNAG/Cr (U/mg)	25.2±30	15.90±11.9	P<0.001**

* P<0.05, ** P<0.001, ^{NS} P>0.05, Data were expressed as mean±SD

Hb: haemoglobin; Hct %: hematocrit; MCV: mean corpuscular volume; RDW: red blood distribution width; Cr: creatinine; FENa: fractional excretion of Na; UNAG: urinary-N- acetyl- beta -D-glucosaminidase.

DISCUSSION

The deleterious effects of iron deficiency anemia (IDA) on the nervous and cardiovascular systems were thoroughly investigated, but the effect of IDA on renal function has not been examined in depth. In the current study, there were no differences between the IDA and control groups regarding serum sodium and creatinine, urine osmolality, urine microalbumin, level Ca/Cr and FENa %. This is in accordance with Ozcay et al [15]. The increased urinary β2 microglobulin is a reliable indicator of proximal tubular damage. Urinary excretion of β2 microglobulin is increased in a variety of diseases that impair proximal tubule function such as iron toxicity and minor or major thalassemia [16]. The results of our study showed that urinary β2-microglobulin was significantly higher in IDA patients than in controls. This is in contrast with the results of a previous citied study [17] which showed that urinary β2 microglobulin levels were not significantly different between IDA patients and controls. Ozcay et al [15] did not assess urinary excretion of β2 microglobulin in patients with IDA and used increased FENa and UNAG/Cr as indicators of renal tubular dysfunction. In clinical practice, the most sensitive indicator of proximal tubular dysfunction is an increased excretion of tubular proteins which typically increases by 3-4 orders of magnitude above the normal [18]. In our study, excretion of urinary β2 microglobulin was comparably higher in IDA group, thus there was a strong evidence of functional impairment of proximal tubular function. This is further supported by the presence of a negative correlation between Hb level and urinary β2 microglobulin level.

We did not find any difference in FENa between the IDA group and control group. Our results are in disagreement with a previously cited study [17] which reported that FENa was significantly higher in the IDA group than in control group.

The results of the current study are not in accordance with Ozcay et al [15] who studied 20 children with IDA and 20 controls and reported a significant difference in FENa between the IDA group and control group; and explained that proximal tubule reabsorbs three times the sodium that the distal tubule reabsorbs [19] and this is why increased FENa reflects mainly proximal tubular damage. Sodium reabsorption is the primary determinant of renal oxygen consumption [20], thus the increased sodium excretion in their anaemic patients may have been due to low cortical tissue oxygenation. From our point of view this could be explained in a different way depending on renal physiology: first of all we now know that the proximal tubule absorbs approximately 80% of

filtered sodium [21], and secondly FENa can be increased solely due to distal tubular dysfunction. Patients with primary hyperaldostrinism type 1, for instance, have increased FENa solely due to impaired sodium reabsorption in the collecting ducts. In fact, their proximal tubular reabsorption is increased. Furthermore, they did not find a correlation between FENa and haemoglobin level and explained this finding by their patients' mild-to-moderate anaemia status and hypothesised that if the patients in the anaemic group had exhibited more severe IDA, they could find a negative correlation between Hb levels and FENa. Our results do not support their hypothesis as inspite of the larger number of IDA patients in our study as we studied 100 patients and 35% of our patients had Hb levels <8 g/dl (severe anaemia) we failed to report any correlation between Hb levels and FENa % in the group with IDA.

Sodium is the primary determinant of intravascular volume and the kidney's task is to maintain this perfusion volume by adjusting sodium excretion. In normovolemia, this means that excretion mirrors sodium intake. Thus, the most likely explanation for the increased FENa in the iron deficient children is that they eat more salt. In fact, their diet has to be different from the controls; otherwise they wouldn't be iron deficient. Presumably, they just consume a diet lower in iron and higher in sodium. This needs to be discussed. Some authors could argue the converse: due to proximal tubular damage, FENa is increased and thus the children eat more salt to maintain volume, but there are good arguments against that: a) in proximal tubule, calcium is absorbed passively along with sodium and impaired proximal tubular sodium reabsorption thus results in hypercalciuria [22] Since calcium creatinine ratio is comparable in both groups there is no evidence for impaired proximal sodium reabsorption. B) Impaired proximal sodium reabsorption is usually compensated for by distal segments. Thus, patients with renal Fanconi syndrome usually have a FENa <1% [21].

Research has shown that NAG, a lysosomal enzyme found in proximal tubule cells, is a reliable indicator of proximal tubular damage [23]. Urinary excretion of NAG is increased in a variety of diseases and disorders that involve impaired proximal renal tubule function. In our study, Hb levels had a negative relation with UNAG/Cr in both groups of patients with IDA and the patients with IDA had a higher mean UNAG/Cr than the controls, indicating proximal tubule damage in the anaemic group. Our results are in accordance with Ozcay et al [15] who found that Hb levels were negatively correlated with UNAG/Cr and the patients with IDA. Our results also agree with Zaki et al [24] who studied UNAG as an index of renal tubular function in elderly people with IDA and found that UNAG levels were significantly higher in IDA than in controls, with significantly higher mean values observed in patients over 70 years than in those ≥ 65–70 years. The results of the current study investigated for the first time the relation between renal tubular function and iron profile in children with IDA and showed that β2 microglobulin and UNAG/Cr were negatively correlated to both serum ferritin and serum iron. These findings could suggest that iron deficiency itself might affect renal tubular function. Even in the absence of anemia.

Mehta et al. [24] assessed renal function in 20 adults with IDA. They found that renal function, as measured by 3-h creatinine clearance was impaired in IDA, and that it improved after 3 days of intravenous iron therapy. Since no significant Hb rise was observed with iron administration, the authors speculated that iron effects at the tissue level might have caused the decreased creatinine clearance. Iron is vital to the function of iron-dependent enzymes including mitochondrial cytochromes, which participate in ATP generation. It has been reported that iron deficiency led to an oxidant-induced damage to mitochondria in rat liver [25]. Recently it was proved that IDA reduced glomerular density, glomerular surface area, and promoted fibrosis. Iron substantially rescued renal growth and development, supporting the critical role of iron in late nephrogenesis [26]. Renal iron deficiency itself might disturb mitochondrial energy metabolism and affect renal tubular function.

CONCLUSION

The results of this study provided evidence that patients with IDA exhibit proximal tubular damage and dysfunction. Further studies are needed to determine the cause(s) of renal tubular dysfunction in IDA, and whether or not those changes are reversible with treatment of anaemia.

Limitation of the study

Follow-up of children during and after iron therapy could have been useful to whether or not renal tubular dysfunction is reversible with treatment of anaemia.

ACKNOWLEDGMENT

The authors would like to thank the patients and healthy subjects who willingly participated in the study.

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