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Evaluation of Hematological Parameters Among Children with Autism in Duhok City

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Abstract

Background and objective: Western studies have linked autism with nutritional deficiencies, particularly those of iron and folate, while such studies are scarce from developing countries. We aimed to assess such deficiencies and their associated hematological abnormalities among Iraqi autistic children.

Methods: In this cross-sectional study 100 diagnosed autistic children, visiting the mental health center in Duhok, Iraq in the period (December 2022-June 2023) were recruited. The records of these patients were reviewed and they had blood counts, serum iron, total iron binding capacity, transferrin saturation, ferritin and folate assayed. Patients were labelled as iron deficient if they had serum ferritin<30 ug/L, and/or transferrin saturation<16%. They were further categorized as anemic or non-anemic based on their hemoglobin levels.

Results: Patients had a median age of 7 years (range 4-13), included 75% males, and 17% with severe autism. Iron deficiency anemia was documented in 1%, while non-anemic iron deficiency in 41%. Serum ferritin, transferrin saturation, and folate were significantly lower in severe cases (P<0.001, 0.007 and 0.035 respectively). Iron deficiency was significantly more frequent in the severe cases (P<0.001), and among preschool children (P=0.009).

Conclusion: Iron deficiency was frequently observed among autistic children, although it was latent in the large majority. Furthermore, iron deficiency and low serum folate were associated with more severe disease.

Key words: Autism, Folate deficiency, Iraq, Iron deficiency

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Introduction

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders characterized by deficits in three developmental areas namely: social interaction, communication and restrictive and repetitive behaviors. The expression of these deficits varies in scope and severity. Autistic symptoms appear in childhood and negatively affect social, occupational, or other domains, and constitute a large burden on patients, their families and society.[1] The estimated global prevalence of autism is about 1:100, though this varies remarkably within and across sociodemographic groups, but it is believed to be increasing with time as public awareness increases among various communities.[2] The etiopathogenesis of ASD is still unknown, though it is proposed that a combination of genetic and environmental factors may be at play.[3] Several prenatal and postnatal risk factors have been linked to the development of autism. The prenatal factors include maternal infections, advanced paternal age, male fetus, obstetric complications, and stressful pregnancy, while the post-natal factors include nutritional deficiencies, and metabolic imbalances.[4] There is increasing evidence of important roles of environmental factors in the etiology of ASD, particularly nutritional deficiencies like those of iron and folate. Iron plays an important role in cognitive, behavioral, and motor development. Reduced iron in the brain may be accompanied by changes in dopaminergic, and serotonergic systems as well as myelination, and thus iron deficiency would affect learning, attention, memory, and psychomotor functions.[5,6] Folic acid on the other hand, is crucial for proper brain functioning and plays an important role in mental and emotional health. Folic acid is essential for nucleic acid synthesis and to methylation of monoamines in many biosynthetic pathways.[7] Several investigators have found a beneficial effect of the folic supplementation during pregnancy on the risk of ASD, while others suggested that in diagnosed ASD it may lead to behavioral and neurological improvements, which suggests a potential role of folic deficiency as a risk factor or aggravator of ASD .[8] Studies on autistic children from developing countries including Iraq, are scarce. In the current study we aimed to investigate the hematological findings in autistic patients attending a main public Mental Health Center in Duhok, assess their iron and folate status, and determine whether any abnormalities in these parameters are associated with autism, age group or disease severity.

Patients and methods

This is cross-sectional study carried out in Kurdistan region of Iraq at the Duhok children and adolescent mental health center in the period between December 2022 and June 2023.A total of 100 patients of autism diagnosed according to criteria laid by Diagnostic and Statistical Manual of mental disorder (V) were enrolled. [9] The diagnosis and assessment of severity was performed by a team of two experienced psychiatrists recruited by the center. The severity was assessed by Indian scale for assessment of autism (ISAA).[10] At the time of enrollment, the records of each patient, demographic data, history of any medications, and any concomitant medical illness were recorded. For inclusion, patients had to be 4 years or older; while those having apparent acute or chronic infections, or were on iron or folate therapies at the time were excluded. Each patient was investigated by full blood count using a hematology autoanalyzer (Swelab, Sweden), and had their serum iron, total iron binding capacity, and serum ferritin assayed using a Cobas c311 Analyzer (Roche, Germany). Transferrin saturation was calculated by dividing serum iron by total iron binding capacity multiplied by 100. For the purposes of this study the patient was considered iron deficient if they had a serum

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ferritin <30 ug/L, and/or a transferrin saturation (TS) < 16%.[11,12] The patients were further categorized as iron deficiency anemia (IDA), if they had hemoglobin < 11 g/dl for preschool children or <12.0 for school children, while they were considered non-anemic Iron deficiency (NAID) if their hemoglobin concentration were above the latter limits.[13] The study was approved by the Kurdistan Higher Council of Medical Specialties and informed consents were obtained from the guardians of the enrollees. Statistical analysis was performed using an SPSS software (IBM corp., SPSS v22, USA). A chi square test was used to assess categorical variables while a student t-test was used for comparing continuous variable between groups. P value of < 0.05 was considered significant.

Results

The 100 enrolled ASD patients had a median age of 7 years, range (4-13 years), and consisted of 75 males and 25 females. They included 31 preschool (<6 years) and 69 and school-aged children (≥ 6 years). When patients were classified according to ISAA ASD severity scoring system, it was found that the majority were mild (57%), while 26% were moderate and 17% were severe ASD.

Table (1) outlines the main hematological parameters among the enrolled patients. Serum ferritin was less than 30 ug/dl and/or transferrin saturation < 16% in a total of 42 patients, who were labeled as the Iron deficient group (ID). The latter group was further categorized based on presence of absence of anemia in association with iron deficiency into non-anemic iron deficiency (NAID) in 41 patients, and iron deficiency anemia in one patient (IDA) (8-year-old boy with hemoglobin of 10.8 g/dl, S. ferritin of 13.7 ug/L, and TS of 12%). Two patients were found to be folate deficient (S. Folate < 3.8 ng/ml). One of the latter patients was in the ID category, while the other was iron replete, but neither patient had anemia (for age), Figure (1).

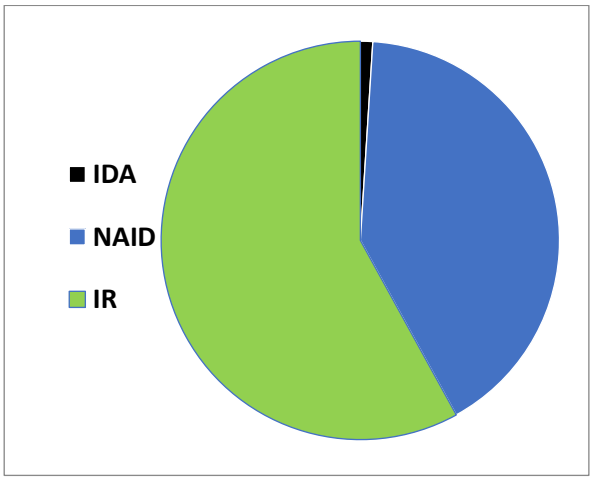

Figure (1): Pie chart showing the relative distribution of iron deficiency anemia (IDA), Non-anemic iron deficiency (NAID) and Iron replete (IR) status among 100 autistic children recruited.

Table (1): The main hematological parameters in 100 ASD patients

Parameter (unit)	Mean (SD)
Hemoglobin level (gm /dl)	12.84 (1.35)
MCV (fL)	78.6 (5.47)
MCH (pg)	26.01 (2.05)
RDW (%)	12.52 (1.38)
S. Ferritin (ug/L)	44.72 (35.27)
S. Iron (ug/dL)	84.4 (46.59)
Total Iron Binding capacity (ug/dl)	348.22 (69.76)
Transferrin saturation (%)	24.3 (10.66)
S. Folate (ng/ml)	12.0 (4.22)

Table (2) outlines the mean hematological parameters (SD) as they relate to disease severity, and it shows that while there were no significant differences in hemoglobin or RDW between severe and mild-moderate

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cases, S. ferritin, folate and TS were significantly lower in severe versus mild-moderate cases (P=0.007, <0.001 and 0.035 respectively)

Table (2): The mean (SD) of some hematological parameters in ASD enrolled patients, categorized according to severity.

ASD Severity	Mean (SD)		P value
	Mild/Moderate	Severe	
Hemoglobin gm/dl	12.89(1.66)	12.78(1.34)	0.846
RDW (%)	12.45(1.37)	12.85(1.43)	0.274
Transferrin Saturation (%)	25.61 (10.72)	18.08(8.05)	0.007
S. Ferritin (ug/L)	49.50(36.68)	21.43(10.39)	<0.001
Folate (ng/ml)	12.39(4.12)	10.02(4.3)	0.035

When the 42 Iron deficient and 58 iron replete ASD patients were categorized according to their disease severity, it was noted that iron deficient cases segregate mostly to the severe category, with 16 out of 17 (94.1%) of the severe cases being iron deficient, while the respective figures for mild/moderate severity were 26 out of 83 (31.3%). This distribution was highly significant with the chance of iron deficiency occurring in the severe category being 35.1 folds (95% CI 4.4-278) higher than the mild/ moderate category (P<0.001).When patients were categorized as preschool or school-aged, it was found that 19/31 (61.3%) preschool children were iron deficient, compared to 23/69 (33.3%) of school children, a distribution which was highly significant (OR 1.84 [CI 1.20-2.84]; P=0.009). While there was no significant difference in the distribution of ASD patients according to severity between preschool and school-aged children (P=0.674).

Discussion

This study is the first study to evaluate the hematological changes in children with ASD in Iraq. The most frequently reported hematological disorder associated with ASD worldwide is iron deficiency. [14] However, the prevalence of iron deficiency in ASD varies in different studies and in different populations. In Turkish ASD children, it was documented that 6.5%-15.5% had IDA, while NAID was noted in 24.1%-32.3%.[13,15] Al-Ali et al identified IDA in 13.3%, and NAID in 6.7% of their Palestinian ASD children, while Gunes et al found IDA in 13% and NAID in 25% of their Italian patients. [16,17] Higher rates of IDA and NAID were reported among Indian ASD patients at 26% and 38% respectively. Lower rates were reported from USA and Australia, with Reynolds et al reporting IDA in 0.5% and NAID in 8% of their USA patients,[18] while Sidrak et al reported respective rates of 3% and 10.5% among Australian patients.[19] The frequencies of IDA and NAID in the current study from Iraq were 1% and 41% respectively. Such variation is at least partly relevant to the definition of iron deficiency, so that while some studies define ID by serum ferritin, others also use S. iron and TS. Moreover, the cut-off points of serum ferritin used vary, with many of the studies using a cut-offs of <10-12 ug/L or < 15 ug/L.[13,20-22] In the current study we used the cut-off point of <30 ug/dl which was reported to be the most sensitive and specific for ID, whether anemia was present or not, and it was strongly associated with absent body iron stores.[11,23] Furthermore, the reliance on S. ferritin as the sole parameter to define iron deficiency has its limitations, since serum ferritin is an acute phase reactant, and to overcome this limitation we included TS as another defining parameter, since TS < 16% is considered as an indicator of inadequate iron supply for erythropoiesis.[12] The inclusion of the latter parameter would cover for any unrecognized inflammatory/infective process at the time of enrollment, which would limit the validity of S. ferritin.[23] However, it is noteworthy that the frequency of iron deficiency (1%) in our

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cohort is lower than most studies worldwide, which may be related to the fact that ASD patients visiting the mental health center in Duhok are a closely monitored group, whose regular monthly attendance is a prerequisite for receiving social security benefits, and thus patients are regularly assessed, and if anemia is discovered, it is promptly investigated and treated. This explains why almost all cases of iron deficiency identified in the current study were latent or non-anemic iron deficiency, whose relative high frequency in the current study is likely to be due to higher ferritin cut-off point and the inclusion of TS as an additional indicator for ID. In the current study, it was found that ID was much more frequent among preschool children at 61.3% compared to school children at 33.3%. This is similar to the observations on Turkish and British autistic children where ID was higher among preschool compared to school children.[13,15,22] This may be explainable by the more restrictive diet in younger Preschool children.[13] The lower intake of iron in diet in preschool autistic children and its increase with increasing age has been documented by some observers.[24] However, and contrary to our observations and the earlier reports, other investigators found that ID was either less frequent or not different between preschool and older Canadian or Italian autistic children.[17,20] The current study also documented that the frequency of Iron deficiency increases significantly with increasing severity of ASD. Such observation is shared by Gunes et al who found higher proportion of IDA in patients with severe versus mild-moderate ASD.[17] Moreover, Dosman et al documented that lower ferritin is associated with more severe communication impairment in ASD.[20] Likewise, Parakash et al documented that severe ASD was more likely to be associated with ID, and severe ASD was associated with lower ferritin, hemoglobin and iron than mild-moderate Indian ASD patients.[25] This is in contrast to observations of Belgic, who argued against a link between severity and low serum ferritin in Turkish autistic children.[15] The association of iron deficiency with autism and its severity in the current study and in several earlier studies in intriguing. One possibility that has been floated over the years, is that a common genetic mechanism for both autism and iron deficiency may be the culprit. A recent mendelian randomized study revealed that genetically predicated serum transferrin levels might be causally associated with the risk of autism, suggesting a potential role of iron deficiency in autism development.[26] Another possibility is related to the fact that iron plays a key role in the dopaminergic, serotonergic CNS pathways, and in myelination of neurons, and that these pathways are involved in autism, and iron deficiency affects these pathways.[27] This concept is supported by the observation that more severe ASD is more likely to be associated with iron deficiency as reported by the current study and by others.[17,20,25]. Some other investigators suggested that malabsorption of iron may be the culprit of ID in autism, but this suggestion has been disputed by the fact that iron therapy would correct iron studies in ID autistic children.[28] Although several or any of the above possibilities may offer an explanation for the association of ID and autism, a more popular plausible explanation is the reduced dietary iron intake, since children with autism often have very restrictive dietary habits. Such highly selective dietary habits are more likely to be encountered in younger than older children.[28] The latter is consistent with our own observations where preschool children were more likely to be ID than older ones. Furthermore, the adequacy and variety of diet may be more compromised in more severe disease, and this is again consistent with our own observations and those of others.[17,20,25]

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Though some observers dispute the concept that dietary inadequacy as the culprit, at least in American autistic children.[18] In this study, we found folate deficiency in two patients, neither of whom were anemic, while we found that the mean folate levels were significantly lower in severe versus mild-moderate autism. The latter observation may be related to the more restrictive dietary habits among the more severe ASD patients. These observations are consistent with previous studies which showed a negative correlation between serum folate and ASD severity.[29] ASD has been linked to abnormalities in folate metabolism, and polymorphisms in folate genes may be implicated in increasing the risk of developing ASD. Furthermore, it was found that a significant proportion of ASD patients have cerebral folate deficiency syndrome mostly due to autoantibodies blocking folate transport into the brain.[30] Furthermore, folate deficiency has been linked to cognitive impairment.[31] The limitations of the current study include the fact that it is a cross-sectional study and not a case control study, and our justification is that the cohort recruited is a high monitored and investigated group, which makes finding a matching control quite difficult. Another limitation is that the study did not include a food survey of the recruited patients, to check for any dietary restrictive practices.

Conclusions:

ASD children had a high prevalence of iron deficiency, although it was latent in the large majority. Furthermore, it was documented that iron deficiency and serum folate levels were significantly associated with disease severity. Due to their crucial roles in various brain metabolic pathways, it may be prudent that Iraqi ASD patients be routinely assessed for their iron and folate status, and that future studies addressing the impact of iron and/or folate supplementation on various aspects of ASD be initiated, particularly in deficient cases. Future studies directed at assessing the impact of iron and/or folate supplementation on various clinical findings in Iraqi autistic children may be warranted, particularly in deficient cases.

Conflict of interest:

None

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