Determination of the Association Between Serum Ferritin and C-Reactive Protein in Children Presented with Iron Deficiency Anemia in Combined Military Hospital Pathology Department Multan

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ABSTRACT

Objective: To determine association between Serum ferritin and C-reactive protein (CRP) in children having iron scarcity anemia.

Study Design: Cross sectional study.

Place and Duration of Study: Pathology Department, Combined Military Hospital Multan Pakistan, from Jun to Dec 2018

Methodology: One hundred and thirty-eight (138) children were consecutively selected as per inclusion and exclusion criteria. Each sample of blood was collected from children for serum ferritin, Blood complete picture (Blood CP) which include hemoglobin level and red blood cell indices and serum sample for C-reactive protein (CRP). Sandwich chemilumine-scence immunoassay technique was used to analyze serum ferritin, Blood CP was analyzed on sysmex and C-reactive protein was analyzed immuneturbidi-metric method.

Result: A total of 138 children were included in our study. Mean age group of our population is 4.5 ± 0.65 years. Out of these 138, 67 (49%) were male children and 71 (51%) were female children. CRP was found negative in 50 (36%) and positive in 88 (64%) children. ANOVA and chi-square test showed association between CRP and anemia was statically significant.

Conclusion: Increased ferritin level, secondary to inflammation may mask an underlying iron inadequacy in children. CRP in children with normal or high ferritin can be helpful in diagnosing iron deficiency. Serum ferritin was found to be positively correlated with CRP in children.

Keywords: Blood complete picture, Iron deficiency anemia, Serum ferritin c-reactive protein.

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INTRODUCTION

In the presence of infection and inflammation, assessment of iron status become challenging and confusing due to presence of acute phase reactants which affect indicator of iron status. This study is planned to enlighten the effects of these acute phase reactants on iron status and determine the impacts of these factor on iron deficiency (ID) in infection-burden settings. In spite of notorious impacts of iron deficiency on health, exact distribution and magnitude of its deficit are unknown. The concentration of hemoglobin is used to asses the anemia but it is neither a specific nor sensitive marker for iron deficiency anemia. For instance, the world health organization Micronutrients database in vitamin and mineral nutrition information system (MDVMNIS), which examined the signs of vitamin and mineral nutrients in the people, had included only the concentrations of haemoglobin but it did not included the indicators of level of iron.

Iron insufficiency is one of the most frequent nutritional deficit and most common anemia is iron deficiency anemia.[1] It remained and it is a matter of great concern in public health in both developed and developing countries. In USA, the deficiency of iron is 15 % and the percentage of anemic patients with iron deficiency is 2%. The incidence of iron deficiency peaks has been observed in early childhood, this is the age at which brain is developing rapidly.[2] Deficiency of iron leads to negative effects on neuro-developmental outcome. Iron also plays pivotal role in metabolism of monoamines, myelin synthesis, and metabolic functions of brain. In literature it is mentioned that animal studies have shown iron deficiency in early postnatal life changed brain development and cognition while in human, iron deficiency has been associated with negative impacts on multiple domains of child development.[3] Iron deficiency in early childhood might continue in adolescence and adulthood in the form of Impairments in cognitive, social, and emotional

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functioning.[4] It is also mentioned in literature that there is improvement in motor and cognitive functions among infants and children with iron supplements.[5]

In 2016, NIH (the National Institute of Health) has conducted a workshop on iron deficiency. The work-shop has reported that decreased level of ferritin in serum is the surrogate marker of iron deficiency.[6] It has also been reported that serum ferritin is an acute phase reactant, therefore use of decreased serum ferritin as an indicator of iron deficiency in concomitant of inflammation is not a good choice. The outcomes of inflammation can lead to wrong examination of malnutrition in children. Not only this, it can also lead underestimation of prevalence of iron deficiency.[7] To address this, C-reactive protein (CRP) has been used as a marker in iron deficiency in children concomitant inflammation.

In Canada, screening program for deficiency of iron and anemia in primary health care has not been recommended. Furthermore iron deficiency or anemia is more prevalent in developing countries like Pakistan.[8] Acute inflammation is also more prevalent in such countries. In previous literature there is no such research in our population so our study will provide useful data for researchers to design more research projects for future.

METHODOLOGY

This cross-sectional, study was conducted in the department of Pathology, Combined Military Hospital Multan, Pakistan, from June to December 2018. The research was permitted by the ethical review committee of hospital. The research included patients from both genders ranging from 1 day to 12 years and having Hypochromic microcytic anemia and non probability consecutive sampling technique was used. Patients already on iron therapy and those diagnosed with iron surplus were excluded. Other exclusion criteria set were based on medical conditions which could possibly affect the ferritin, such as hemoglobinopathies or any bleeding disorders. After taking Informed consent from parents or attendants present and past medical/surgical history, 5mL of venous blood was drawn; 3mL was put in gel tubes for the determination of serum ferritin and CRP and 2 ml whole blood sample in EDTA tube for Blood CP. Serum ferritin was analyzed by the Electrochemiluminesence Immunoassay on Roche autoanalyzer Cobas e 411, Serum CRP Quantify by Immunoassay method Blood CP on Sysmex haematology autoanalyzer. Those Individuals whose blood CP showed hypochromic microcytic blood picture with normal TRBC, most probably of ID were included in the study and their ferritin and CRP was done. All the data was entered and analyzed by SPSS version 21. Chi square test was applied establishing an association of iron deficiency anemia with serum ferritin and CRP, *p*-value of <0.05 was considered as significant.

Operational Definition:

IDA = Hb <10 gm/dl, TRBC <4 x10^{12}/L, MCV <60 fl, MCH <26pg, MCHC 28 g/dl

Ferritin <12 µg/L

The WHO-defined Ferritin concentration cut off in the presence of inflammation of <30 µg/L.

RESULT

A total of 138 children were included in our study. Mean age group of our population is 4.5 ± 0.65 years. Out of these 138, 67 (49%) were male and 71 (51%) were female. CRP was found negative in 50 (36%) and positive in 88 (64%) children. Mean values of Hb, ferritin, HCT, MCV, MCH, MCHC, RDW and TRBC were 8.9 ± 2.2 g/dl, 48.3 ± 2.89 ug/L, 30.12 ± 2.6, 66.09 ± 9.6 fl, 20.72 ± 11.6 pg, 21.32 ± 1.9 g/dl, 17.27 ± 3.7 and 4.74 ± 0.7 respectively (Table-II).

Table-I: Chi- Square test shows association of IDA with CRP.

Variables	Value	*p*-value
Pearson Chi-Square	125.298a	0.000
Continuity Correctionb	121.190	0.000
Likelihood Ratio	151.809	0.000
Fisher's Exact Test	-	0.000
Linear-by-Linear Association	124.390	0.000
No. of Valid Cases	138	0.000

Table-II: Variables with mean and SD values.

Varaibles	Hemoglobin g/dl	HCT ug/L	MCV fl	MCH Pl	MCHC g/dl	Ferritin Ug/dl	CRP
Mean ± SD	8.9 ± 2.2	30.12 ± 4.6	66.1 ± 9.2	20.7 ± 11.9	31.3 ± 9.6	48.3 ± 2.9	0.6 ± 0.3

Kolmogorov test was applied which showed *p*-value more than 0.05 which indicate data is parametric. Moreover, one-way ANOVA showed a statistically significant between CRP and anemia (Table-III).

Table-III: ANOVA test shows association between CRP and Anemia.

Variables	*p*-value
Hemoglobin	0.000
Anemia	0.000

Chi square was applied between the IDA and the dependent variables, namely ferritin and CRP. It is also notable that ferritin had a strong positive correlation with CRP.

DISCUSSION

Determination of iron status and inflammation, need test which should be user friendly, cost effective, easily available and can be applied and interpreted globally. The test is standardized, so that its result can be compared over time and across population.[9] It is also important for an ideal marker that its level should be comparable with different causes e.g. infection, autoimmune diseases, cancer or obesity, of anemia and determine relationship between iron status and inflammation or infection.[10] Among different diseases, liver disease, in particular, has directly elevated the concentration of serum ferritin (SF) regardless, inflammation through damage of hepatocytes and discharge of contents present inside the cells. Serum ferritin concentration is also raised in obesity due hepatocytes damage due to non-alcoholic steatohepatitis but indicators of infection or inflammation remain within normal limit.[11]

Measurement of indicators of inflammation while determining iron status at population level has been enlightened by the biomarkers indicating the Inflammation and Nutrition Determinants of Anemic patients (BRINDA). Recent studies in children have been identified that indicator of inflammation or infection change by socio-demographic, settings and information of morbidity alone cannot reliably detect or asses the infection or inflammation, thus has suggested the importance of measurement of biochemical markers of subclinical inflammation.[12] Concentrations of CRP might have importance because it shows different phases of the acute phase reactant (APR) that vary from acute inflammation (e.g., rapid onset within 1 h) to chronic infection (e.g., rising after 24 h and lasting 4-5 days).[13]

The WHO has recommended serum ferritin to determine iron scores in population surveys. Deficiency of iron is one of the important public health problems, which is more prevalent in childhood and leading to detrimental consequences to cognitive, social, emotional and motor development.[14] Iron deficiency in nonanemic children is known as latent anemia and can be identified before developing anemia. Serum ferritin is a cheaper, easily available and less invasive test, has high specificity for iron deficiency.[15] In the presence of inflammation, infection and chronic diseases, serum ferritin is either within reference interval or high which leads to misdiagnosis of iron deficiency anemia. WHO/UNICEF has recommended inflammatory markers like CRP to determine the actual body iron status.[16]

In our study children with iron deficiency anemia who has normal and high ferritin due to underlying inflammatory conditions diagnosed by high CRP value and showed a positive correlation between CRP and serum ferritin.[17]

A study was conducted in Peshawar, Pakistan in obese and overweight children with iron deficiency anemia.[18]The level of serum ferritin was found higher in these obese, overweight people, because of presence of generalized inflammation in them. Due to this reason, using serum ferritin level as a marker of Iron deficiency anemia in children associated with inflammatory conditions is controversial.[19]

Decreased plasma ferritin level is the best marker of iron deficiency. Ferritin concentration is increased in the presence of inflammation. CRP is used to determine the status of iron in body.[20] In our study, serum ferritin level of children with inflammatory conditions was higher compared to children without inflammation. Actually, in inflammatory conditions, iron regulatory hormone, hepcidin inhibit iron transport in circulation. Hence, endogenous iron is used in erythropoiesis rather dietary iron.[21] This leads to increased serum ferritin level. Similar results were drawn by Knowles *et al*, where the concentration of serum ferritin level in children with inflammation was elevated in comparison with children without inflammation.[22]

Ferritin was not an actual indicator of basic iron deficiency anemia in our targeted study population. Positive correlation is observed between serum ferritin and CRP in children with iron deficiency anemia associated with inflammatory conditions. This study will be helpful in diagnosis of iron deficiency anemia in children associated with inflammation and early management to prevent complications due to iron deficiency. However, we recommend more studies

with large sample size, in order to draw a more logical inference.

RECOMMENDATIONS

A multicenter prospective study should be commenced to ratify causality between iron deficiency anemia with inflammation and CRP in different ethnic groups of Pakistan.

CONCLUSION

High ferritin level secondary to inflammation may mask an underlying iron deficiency in children. CRP in children with normal or high ferritin can be helpful in diagnosing iron deficiency. Serum ferritin was found to be positively correlated with CRP.

Conflict of Interest: None.

Author's Contribution

WH: Literature review, HI: Data collection, MY: Manusricpt data, NRH: Sample collection, OA: Manuscript writing, QUA: Statistics of article.

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