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COMPARATIVE STUDY OF ACUTE PHASE PROTEINS IN CASE OF ANEMIA OF CHRONIC DISEASE (ACD) AND IRON DEFICIENCY ANEMIA (IDA) AND ITS RELATIONSHIP WITH ERYTHROPOIETIN

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ABSTRACT

Acute phase proteins play a significant role in anemia of chronic disease and thought to be beneficial in maintain homeostasis. In both the condition, anemia of chronic disease as well as iron deficiency anemia alteration in iron homeostasis takes place. The study includes patients of pulmonary tuberculosis patients (ACD), Iron deficiency anemia (IDA) patients and control subjects. Serum CRP, Serum Haptoglobin (HPT), SerumTransferrine (TRF) and Serum Erythropoietin (EPO) levels were estimated in both the study groups. These values were compared to control subjects. The result of the present study reveals that alteration of acute phase protein takes place in the study groups which leads to disturbance in iron metabolism, resulting anemia. However the changing pattern in Acute Phase Proteins in Anemia of Chronic Disease (ACD) and Iron Deficiency Anemia is different (in ACD, Serum level of CRP, HPT,FRT increases whereas serum level of TRF decreases. But in case of IDA Serum level of CRP and TRF increases whereas serum level of FRT decreases and there was no significant change (p > 0.05) observed in Serum Haptoglobin level, when this value was compared with the value of controls).

KEY WORDS

Acute Phase Proteins, Anemia of Chronic disease, Iron deficiency anemia, Erythropoietin, dysregulation of iron metabolism

INTRODUCTION

Acute Phase Proteins are a class of diverse Proteins whose blood plasma concentrations increase (positive acute phase protein), or decreases (negative acute phase protein) during the response to inflammation in the acute phase. They are produced within a few hours by the liver, responding to inflammatory cytokines such as IL-1, TNF-α and in particular IL-6.[1, 2, 3] The function of positive APPs is important in opsonization and trapping of microorganisms and their products, in activating the complement system, in binding cellular remnants like nuclear fractions, in neutralizing enzymes, scavenging free hemoglobin and radicals, and in modulating the host's immune response. It has been reported that Acute Phase Protein, hepcidin, synthesized the liver is increased during inflammation which leads to disturbance in iron homeostasis, resulting anemia. The aim of the present study is to compare the abnormalities in Acute Phase Proteins and Erythropoietin in Anemia of Chronic Disease (ACD) and Iron Deficiency Anemia, and to find out what is the probable cause of anemia in such patients?

REVIEW OF LITERATURE

The most common type of anemia observed is Iron-Deficiency Anemia (IDA), followed by thalassemia and anemia of chronic disease (ACD).[4] Tuberculosis is a

chronic infectious disease, so anemia of inflammation may contribute significantly.[5] However, anemia of inflammation is considered a major contributor to anemia observed in developing countries.[6] It has been reported that a small peptide hormone, hepcidin might be involved in the pathogenesis of Anemia of Chronic Disease (ACD).[7] In addition to its effect on iron metabolism, hepcidin might contribute to EPO resistance by directly inhibiting erythroid-progenitor proliferation and survival.[8] C - reactive protein (CRP) an acute phase protein, recognizes foreign pathogens and damaged host cells and initiates their elimination.[9] Other Scientist reported that CRP is a modulator of innate and adaptive immunity. Depending on the circumstances, CRP may enhance or dampen inflammation.[10] Haptoglobin (HPT) are acute phase reactants and bind to free hemoglobin released from destructed red blood cells and form Haptoglobin-Hemoglobin complexes which are removed from circulation by the liver and thus it helps in recycling of iron part of hemoglobin.[11] Transferrin- receptor expression is negatively affected by inflammatory cytokines so the level of soluble TfR proceeded at the normal level.[12] It has been reported previously that in the setting of anemia, serum ferritin is the most sensitive lab test for Iron Deficiency Anemia.[13] Erythropoietin (EPO) is a glycoprotein hormone first purified from human urine.[14] It stimulates the production of Red Blood Cells (RBCs) by stem cell in Bone Marrow. Produced mainly by the kidneys, it is released in response to decreased levels of oxygen in body tissue. Anemia in pulmonary tuberculosis may also occur as a consequence of chronic inflammation, and without apparent loss of blood or bone marrow suppression.[15] Blunted responses of erythropoietin due to release of tumor necrosis factor or other cytokines have been observed.[16]

Insufficient endogenous production of erythropoietin (EPO) is probably one of the pathogenic mechanism of the Anemia of Chronic Disease (ACD.[17] In vitro and In vivo evidence suggests that hypoxia and anemia are the most important stimuli of increased EPO production.[18] Inverse correlation between serum EPO levels and red blood cell mass has been reported earlier.[19]

MATERIAL AND METHOD

The present study was conducted in Department of Biochemistry in Shri Aurobindo Institute of Medical Sciences, Indore during March 2008 to March 2010. The study includes 101 subjects in the age groups of 20 to 58 years including 35 control subjects selected from staff and students of SAIMS Medical College, Indore and 30 Patients of Iron deficiency Anemia (IDA) and 36 patients of Anemia of chronic disease (ACD) were selected from the Out Patient Department (OPD) of Medicine in Shri Aurobindo Institute of Medical Sciences, Manorma Raje Tuberculosis Hospital & from different DOTS Centers of Indore. In Anemia of chronic disease patients, only newly diagnosed Pulmonary Tuberculosis Patients falling in DOTS (CAT I) were selected.

Inclusion Criteria for selection of patients (i) Anemic Patients were selected as per WHO criteria (for Male < 13gm/dL & for Female < 12gm/dL) and on the basis of RBC Picture.[20] (ii) IDA Patients were selected on the basis of Serum Ferritin level (< 50 ng/mL). Prior to start of study Informed consent was taken from each subject, and then they were enrolled in the study. The Ethical Committee of SAIMS Medical College, Indore approved the study.

Method

Blood samples of all the subjects were analyzed for hemoglobin by automated cell counter (Sysmex KX-21), Serum C- reactive protein by the method of Votila M et al 1981)[21] serum ferritin by the method of Ronald H et al[22] Serum Haptoglobin and Serum Transferrine estimated Turbidimetry by kit method (Giesse Diagnostics) and serum erythropoietin (EPO) by the method of Garcia J.F (1982)[23] by using kit acquired from DRG International Inc. USA. Statistical Analysis: Unpaired t- test were used for statistical assessments with SPSS Version 10 to evaluate mean levels of variables in study groups. Values were expressed as Mean ± SD.

RESULTS & DISCUSSION

Table 1: COMPARISION OF PARAMETERS BETWEEN CONTROL GROUP & ACD

Parameters	Control (35)	ACD (36)	t-value	p-value
CRP (µg/mL)	2.95 ± 0.72	37.99 ± 7.21	28.58	< 0.001[**]
HPT (mg/dL)	263.48 ± 40.25	375.66 ± 67.46	8.47	< 0.001[**]
Ferritin (ng/mL)	161.31 ± 40.2	385.58 ± 80.08	14.84	< 0.001[**]
Transferrin (mg/dL)	267.97 ± 33.34	192.55 ± 18.1	11.89	< 0.001[**]
EPO (mU/mL)	25.77 ± 3.23	40.44 ± 11.88	7.05	< 0.001[**]

Values are in Mean ± SD   **highly Significant p < o.oo1   **DF = 69**

Table 1. In biochemical parameter the increase in the level of CRP, HPT, Ferritin & EPO were found to be highly significant ($p < 0.001$). Transferring level was decreased and these parameter was statistically highly significant ($p < 0.001$) when these values were compared with control group.

Table 2: COMPARISION OF PARAMETERS BETWEEN CONTROL GROUP & IDA

Parameters	Control (35)	IDA (30)	t-value	p-value
CRP (µg/mL)	2.95 ± 0.72	9.93 ± 2.87	13.86	< 0.001[**]
HPT (mg/dL)	263.48 ± 40.25	261.9 ± 35.8	0.166	> 0.05
Ferritin (ng/mL)	161.31 ± 40.2	35.96 ± 6.95	16.84	< 0.001[**]
Transferrin (mg/dL)	267.97 ± 33.34	371.86 ± 35.64	12.13	< 0.001[**]
EPO (mU/mL)	25.77 ± 3.23	61.83 ± 8.4	23.45	< 0.001[**]

Values are in Mean ± SD   **highly Significant p < o.oo1   p > 0.05 = Non significant   **DF = 63**

Table 2. In **biochemical parameter** the increase in the level of CRP, Transferrin, & EPO were found to be highly significant ($p < 0.001$). Ferritin level was decreased and these parameters were statistically highly significant ($p < 0.001$). Significant change in the level of HPT was not recorded ($p > 0.05$) when these values were compared with control group.

Table 3 COMPARISION OF PARAMETER BETWEEN ACD & IDA

Parameters	Control	ACD		IDA	
CRP (µg/mL)	2.95 ± 0.72	37.99 ± 7.21	↑	9.93 ± 2.87	↑
HPT (mg/dL)	263.48 ± 40.25	375.66 ± 67.46	↑	261.9 ± 35.8	↓
Ferritin (ng/mL)	161.31 ± 40.2	385.58 ± 80.08	↑	35.96 ± 6.95	↓
Transferrin (mg/dL)	267.97 ± 33.34	192.55 ± 18.1	↓	371.86 ± 35.6	↑
Erythropoietin (mU/mL)	25.77 ± 3.23	40.44 ± 11.88	↑	61.83 ± 8.4	↑

Table 3. The above table showed increased (↑) and decreased (↓) level of biochemical parameters in comparison to control subjects. In both the study groups (ACD & IDA), Erythropoietin level was increased but the level was more raised in IDA subjects.
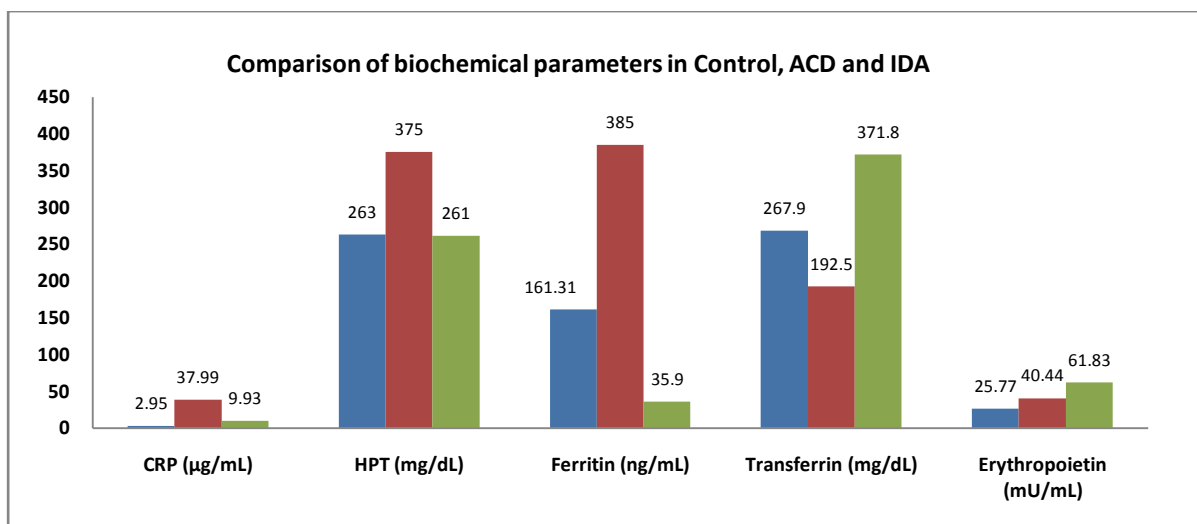
CHART 1: Comparison of Biochemical Parameter value in Control, ACD and IDA subjects

DISCUSSION

Pulmonary tuberculosis is a chronic infective disease occurring predominantly in socio-economically deprived populations. Therefore, both anaemia of inflammation as well as iron deficiency may coexist in pulmonary tuberculosis. In the present study majority of ACD patients had normocyticnormochromic anemia and few cases were of normocytichypo chromic anemia. In Iron deficiency anemia (IDA) patient's subnormal hematological picture is due to low body iron store which influenced Hb synthesis adversely. The blood picture of IDA patients represents hypochromicmicrocytic pattern. Similar findings were reported earlier.[24] In ACD patients a highly significant rise was noted in serum levels of C-reactive proteins (CRP), Haptoglobin (HPT) and Ferritin (FRT) while serum level of transferrin (TRF) was significantly decreased as compared to control group (Chart 1 & Table 1). This finding is consistent with the earlier finding.[25, 26] In pulmonary tuberculosis ferritin synthesis is stimulated by the inflammatory process.[27,28,29] Earlier report suggested that certain acute phase proteins: α- 2 macroglobulin, AAGP, CRP, Ceruloplasmin and Haptoglobin (HPT) were significantly increased in tuberculosis except transferring.[30]. Finding of other scientist suggested that a decrease in concentration of transferrin with severity of disease in pulmonary tuberculosis.[31] In the present study the change in acute phase protein levels in case of ACD patients might be due to increase level of proinflammatory cytokine levels during infection. Ferritin synthesis is stimulated by inflammatory process regardless of iron status, serum ferritin, a non invasive indicator of iron store is an acute phase response. In fact this acute phase response is thought to be beneficial to the organism by preventing microbial growth and helping to restore homeostasis. In Anemia of chronic disease (ACD) patients, highly significant rise was noted in serum levels of EPO as compared to control group. The increase in EPO level in ACD patients might be due to increased level of proinflammatory cytokines & acute phase proteins which leads to blunted erythropoietin resistance (Table 1 & Chart 1). Raised level of CRP is a marker of inflammation which causes blunted erythropoietin resistance in Anemia of chronic disease (ACD).The data based on the result of the present and previous study suggest that blunted erythropoietin resistance occurred during infection with rise in acute phase protein level mainly CRP in anemic pulmonary tuberculosis patients.

In Iron deficiency anemia (IDA) patients CRP & TRF were significantly increased, Ferritin (FRT) decreased while change in level of Haptoglobin was non significant (P > 0.05). The decreased level of serum Ferritin level, in Iron deficiency anemia (IDA) was due to the depletion of stored iron & poor nutrition (Table 2 & Chart 1). In IDA patients our findings are consistent with the earlier finding.[32, 12]

In patients of IDA fall in serum iron level causes reduction in ferritin concentration in blood is considered to be a specific indicator of body iron stores.[33, 34]

Serum TfR measurement is a reliable index of iron depletion and importance in the diagnosis of iron-deficiency anemia.[32, 12]

Highly significant rise of serum level of EPO was observed in Iron deficiency anemia as compared to control (Table 2). Since Iron deprivation stimulates hypoxia, a most potent stimulus to increases EPO production and is a compensatory mechanism to restore iron in IDA patients. . In Iron deficiency anemia (IDA) patients, low serum iron and Hb causes hypoxia that stimulates EPO production. Moreover sub clinical infection in IDA patients increases CRP and other acute phase proteins. This finding is the consistent with earlier study.[17] The report of earlier finding suggested that for any hemoglobin level, the response of erythropoietin was significantly higher in anemic patients with iron deficiency. [35, 36] The results of the present study suggest that, in both the study groups (ACD & IDA), Erythropoietin level was increased but the level was more raised in IDA subjects, when these values were compared with control. Estimation of Serum Ferritin and Transferrin level is essential for discriminate between ACD with IDA (Table 3).

CONCLUSION

The present study reveals that inflammation and acute phase response interact with iron metabolism. The alteration in Acute Phase Protein during infection is the major leading cause of anemia in such patients. However the changing pattern in Acute phases Proteins in ACD and IDA is variable.

Acknowledgement

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