Inflammatory Markers are Elevated in Eisenmenger Syndrome

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ABSTRACT

Background: Inflammatory mechanisms have an established role in the pathophysiology of IPAH and several subclasses of adult associated PAH. In the current study, we sought to assess the degree of involvement of systemic inflammation as reflected by circulatory levels of high-sensitivity CRP (hs-CRP), IL-6, IL-2, Ceruloplasmin, Ferritin in patients with ES.

Methods: 21 patients with severe PAH with preexisting congenital heart disease with bidirectional or reversal of shunt and 20 cases with cyanosis without PAH were selected for the study. Patients had been assessed with transthoracic echocardiography, if required diagnostic catheterisation for shunt estimation. Analysis of the serum ferritin, ceruloplasmin, and hs-CRP, IL-2, IL-6 levels were done in both cases and controls.

Results: Both the control and ES patients showed higher levels of IL-2 (40.74±27.52 vs 17.91±15.52, p value 1.041e-07, significant) and IL-6 (20.62±14.94 vs 10.98±12.5, p value 3.161e-05, significant) but the ES patients showed significantly higher than the control subjects. Significant correlations were not observed with hsCRP (1.02±2.54 vs 0.71±1.26, p value=0.1709, non-significant), ferritin (438±914 vs 408±1529, p value=0.4407, non significant) or ceruloplasmin (21.9±19.42 vs 21.44±11.72, p value=0.2075, non significant). Hb% was high in both cases and control, though it was more elevated in eisenmenger group (21± 2.88 vs19.56± 2.84, p value 0.001014, significant). It was also observed that Hb%, IL-2, IL-6, CER and hsCRP were significantly elevated in ASD variety of eisenmenger patient than other variety.

Conclusions: Inflammation may have some pathophysiologica role in eisenmenger syndrome like other cardiovascular diseases.

Keywords: Pulmonary hypertension, eisenmeger syndrome, hs-CRP, interleukins, ceruloplasmin, ferritin.

INTRODUCTION

Eisenmenger syndrome is an acquired form of pulmonary arterial hypertension and arises on the basis of congenital heart disease with a systemic-to-pulmonary shunt resultant into bidirectional or reversal of shunt. Hemodynamically, Pulmonary arterial hypertension (PAH) was defined by a mean pulmonary artery pressure (PAP) of more than 25 mmHg at rest or more than 30 mg during exercise and Eisenmenger syndrome (ES) is defined as an elevation of the pulmonary vascular resistance to 12 Wood Units or to a pulmonary-to-systemic resistance ratio equal to or greater than 1.0.[1] Endothelial dysfunction and smooth muscle proliferation result from the changes in flow and pressure, increasing the pulmonary vascular resistance. The cellular and molecular mechanisms remain fully uncharacterized, representing pathways of inflammation, cell proliferation, vasoconstriction, and fibrosis. Despite considerable recent advances, the complex pathophysiology of these vascular changes remains incompletely understood. Inflammation is thought to play a key role in the pathogenesis of various forms of pulmonary artery hypertension (PAH), especially experimental models of PAH and PAH associated with connective tissue disease (CTD) and human immunodeficiency virus (HIV) infection[2].
pulmonary vascular pathobiology of ES resembles that described for idiopathic pulmonary artery hypertension (iPAH), with medial thickening and plexiform lesions in severe cases [3]. Plexiform lesions are rich in macrophages, T and B lymphocytes, and dendritic cells[4]. Markers of inflammation, including interleukin-1b (IL-1b), IL-6, and P-selectin, are elevated in iPAH[5,6]. Elevated levels of IL-6 predicts poor 5-year survival in iPAH[6]. A greater understanding of the mechanisms behind the development of PAH remains crucial. Current therapies are inadequate: anticoagulation, digoxin, oxygen, and diuretics, as well as newer targeted treatments such as prostanoids, endothelin-1 antagonists, and phosphodiesterase inhibitors, may offer symptomatic improvement or retard the short-term progression of the disease. Evidence of long-term benefit of any treatment in PAH, however, remains elusive, and interest into new therapeutic strategies is high[7]. In the current study, we sought to assess the degree of involvement of systemic inflammation as reflected by circulatory levels of high-sensitivity CRP (hs-CRP), IL-6, IL-2, Ceruloplasmin, Ferritin in patients with ES.

MATERIAL AND METHODS:

This Case Control study was carried out from January, 2017 to December, 2017 in single centre after ethical committee approval. After taking written informed consent 21 patients with severe PAH with preexisting congenital heart disease bidirectional or reversal of shunt and 20 cases with cyanosis without PAH were selected for the study. All the patients underwent detailed clinical evaluation, routine biochemical investigations including hematologic, renal, and hepatic function testing; chest X-ray; 12-lead electrocardiography, pulse oxymetry and detailed echocardiography. We used the NYHA classification for assessment of functional class, pulse oxymetry at rest. A total of 3 patients underwent right and left heart catheterization. The decision to proceed for cardiac catheterization was at the discretion of the treating physician. Two patient of VSD and one patient of ASD underwent cardiac catheterization. Patients and control subjects who had an infection, fever or history of infection in the previous month or other chronic disease were excluded from the study. Initially, we planned to recruit 50 patients and 50 control subjects as a sufficient number to prove the hypothesis. However, due to constraints of the time frame for the running samples, we ultimately had only 21 patients in the study. Blood (2 cc) two sample was taken from the patients and control group using a peripheral vein for analysis of the serum ferritin, ceruloplasmin, and hs-CRP, IL-2, IL-6 levels. After collection, the blood samples were centrifuged at 5000 rpm to separate the serum, which then was stored in Eppendorf tubes and stored in deep freeze (~70 degrees Celsius). Serum IL-2 and IL-6 levels were measured by enzyme linked immunosorbent assay (ELISA) using BIORAD kit (Rabbit anti Human interleukin-2, Interleukin-6). Values were expressed as pg/mL. Serum hs-CRP, ferritin ceruloplasmin levels were measured with an immunonephelometric method and expressed as mg/dl and ferritin as ug/L.

STATISTICAL ANALYSIS

Statistical analysis was performed using 3.4.3 version of R software. Continuous variables are expressed as mean ± SD and categorical variables as number and percentage. For uneven distributed variables Welch Two Sample t-test was used and for continuous variables and the fixed model effect of ANOVA for categorical variables in comparisons between groups. For all analysis 99 percent confidence interval and p value less than 0.01 considered significant.

RESULTS

Out of total 21 eisenmenger patients, 11 were male (52.38%) and 10 were female (47.62%). 20 patients with cyanotic heart disease without eisenmenger were taken as control. Mean age of the patients was 28 ± 16.98 years. Mean baseline saturation (SaO2) was 88.6 ± 4.8% (range- 72-94%) and mean haemoglobin was 15.1 ± 2 gm/dl (range- 12-20 gm/dl). In this study, 5(23.8%) patients had an atrial septal defect (ASD), 7 patients (33.3 %) had a ventricular septal defect (VSD), 5 patients (23.8 %) had a patent ductus arteriosus, 3 patients(14.2%) had complete AVSD and only one patient(4.7%) had Truncous Arteriosus. A total of 10 patients (47.6 %) were in WHO functional class 2, 9 (42.8%) patients were in WHO functional class 3 and the remainder (9.5%) were in WHO class 4. The ES patients showed significantly higher levels of IL-2 (40.74±27.52 vs 17.91±15.52,p value 1.041e-07, significant) and IL-6 (20.62±14.94 vs 10.98±12.5, p value 3.161e-05, significant) than the control
subjects. Significant correlations were not observed between hsCRP in ES with control (1.02±2.54 vs.0.71±1.26, p-value = 0.1709, non-significant). Ferritin levels were not also significantly different (438±914 vs. 408±1529, p-value 0.4407, non-significant) in ES and control. Serum Ceruloplasmin also failed to show significant difference (21.9±19.42 vs 21.44±11.72, p value 0.2075, non significant) in case and control. The ES patients showed significant elevation of Hb% than control (21± 2.88 vs.19.56± 2.84, p value 0.001014, significant) though there was elevation of Hb% level in both the groups. It has been observed that Hb% was significantly raised in ASD variety of eisenmenger patient than others. IL-2, IL-6, CER and hsCRP were significantly elevated in ASD variety of eisenmenger patient than others variety.

**DICUSSION**

In this study found that IL-2 and IL-6 were elevated in the ES patients compared with the control subjects. Other inflammatory markers including hsCRP, Ceruloplasmin, Ferritin are not elevated in ES than control in this study. Another important observation in this study Hb% is elevated in both case and control but there is significant elevation in Eisenmenger patients than other cyanotic patient. All inflammatory markers investigated in this study are elevated significantly in ASD Eisenmenger syndrome. Inflammation plays an important role in various forms of PAH, including iPAH and PAH associated with connective tissue disorders and HIV infection and in experimental animal models (e.g. monocrotaline-induced PAH).[2] Cool et al.[8] reported that in patients with scleroderma-related PAH, mononuclear inflammatory cells surround vascular sites of plexiform growth but not in uninvolved vessels or extravascular lung structures. In addition, inflammatory infiltrates are observed in plexiform lesions in the lungs of patients with severe iPAH.[9] The resemblance in pathologic anatomy of patients with iPAH may suggest similarity in pathophysiology and underscores a possible role for vascular inflammation in ES. Pentraxin 3 is one of the long pentraxins, synthesized by smooth muscle cells, endothelial cells, fibroblasts and other immune cells at sites of inflammation. Pentraxin 3 plays a key role in the regulation of cell proliferation and angiogenesis,(10,11) Cemsit Karakurt et al. Found Serum pentraxin 3 and high sensitive C reactive protein (hs-CRP) levels in children with severe pulmonary arterial hypertension (PAH) secondary to untreated congenital heart defects. Tamura et al.(13) proposed human pentraxin 3 as a novel biomaker for the diagnosis of pulmonary hypertension. They compared Pentraxin 3 levels were significantly elevated in 50 PAH patients, 27 with idiopathic PAH, 17 with PAH associated with connective tissue disease and six with congenital heart disease.(13) Most of their patients selected for this study were patients with severe pulmonary arterial hypertension related to congenital heart disease. They found that pentraxin 3 levels were significantly increased in the PAH group. They consider that inflammation has an important role in severe paediatric pulmonary hypertension related to congenital heart disease, like connective tissue related PAH. Increased serum levels of IL-1 beta and IL-6 have been demonstrated in severe iPAH, suggesting a role for pro-inflammatory cytokines in iPAH.[14,15] Similarly, in patients with chronic obstructive pulmonary disease (COPD), increases in pulmonary arterial pressure have been associated with higher serum levels of CRP and TNF-α, raising the possibility of a pathogenetic role for low-grade systemic inflammation in the pathogenesis of PAH in COPD patients.[15,16] Thus, inflammatory pathways involving chemokines, cytokines, and growth factors are likely to play a major role in the vascular remodeling associated with PAH due to any cause. [2,17] Apart from hs-CRP, significant elevation of IFN-c was observed in ES patients. Both IL-2 and IL-6 were numerically higher but did not differ significantly from control levels. These results are in contrast to cytokine profiles in iPAH patients, wherein significantly elevated levels of IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, and TNF-α compared with the levels of normal control subjects .[14] In fact, the levels of IL-2, IL-6, IL-8, IL-10, and IL-12 were predictors of survival in a cohort of iPAH patients .[14] However, IFN-c levels were not elevated in iPAH patients. In Ramakrishnan S. et. al. study, ES patients showed a significant elevation in hsCRP (2.99 ± 3.5 vs 1.1 ± 0.9 mg/dl; p = 0.002) and IFN-γ (41.3 ± 43.6 vs 10.4 ± 6.9 pg/ml; p < 0.001) levels. The levels of IL-2 and IL-6 also were elevated but did not differ significantly from those in the control subjects. This was consistent with our present study. They also showed that the patients with hs-
CRP levels higher than 3 mg/dl were significantly older (28.9 ± 10.6 vs 21.5 ± 9.8 years) and had a significantly shorter 6-min walk distance (421.5 ± 133.2 vs 493.3 ± 74.8 m). To date, three major pathways have been identified in the pathophysiology of PAH and serve as target for PAH-specific treatment: (i) endothelin pathway, (ii) nitric oxide pathway, and (iii) prostacyclin pathway. Currently, the strongest evidence for the use of Endothelin receptor antagonists (ERAs) in PAH-CHD is from the Bosentan Randomized Trial of Endothelin Antagonist Therapy-5 (BREATHE-5) and its 40-week open-label extension study. 54 ES patients, bosentan significantly improved exercise capacity, hemodynamics, and functional class,[19,20] independently of the location of the septal defect and without compromising oxygen saturations.[18] Favorable long-term results from several studies have thereafter supported its use in PAH-CHD patients, especially ES.[21,22,23] SERAPHIN trial, a RCT including 62 PAH-CHD patients with closed defects (8% of the study population) demonstrating a reduced morbity and mortality with macitentan.[24] The results from the Macitentan in Eisenmenger Syndrome to Restore Exercise Capacity (MAESTRO) trial in which 220 ES patients were randomized to assess the effects of macitentan on exercise capacity compared to placebo are expected this year. SUPER-1 trial including 18 PAH-CHD subjects with closed defects (6% of the total study population), sildenafil treatment for 12 weeks led to an increase in exercise capacity and improvement in hemodynamics.[25] Similar favorable results of tadalafil have been reported in subgroup analyses of the Pulmonary Arterial Hypertension and Response to Tadalafil (PHIRST) trial, [26] a preliminary study of 16 ES patients,[27] and a crossover RCT in 28 ES patients. [28] In a prospective study with inhaled iloprost (Prostacyclin analogue) in 13 ES patients[29], 24 weeks of therapy led to improvements in exercise capacity and quality-of-life, though hemodynamics did not improve. In the GRIFFON trial, Selexipag (Prostacyclin analogue) definitely holds promise to expand treatment options by targeting a previously underutilized pathway, composite end point of death or PAH-related complications was significantly lower. Inflammatory pathways may be potential targets for therapeutic intervention in PAH. So, it can be said that our study was consistent with other studies that have been conducted on pathophysiology of pulmonary hypertension and eisenmenger syndrome. We had some limitations in our study. Sample size was not large, so power of the study was not strong, study was done in a very short period of time, cases were not followed up, control groups were not age matched and all case were not undergone catheterisation or reversibility. It was a single centre study, so study population may not be real reflection of the actual demography of the disease. The inflammatory parameters used in this study may elevate in inflammation but opposite is not true.

CONCLUSIONS

Inflammatory markers, including IL-2, IL-6 were raised in both in case and control group but, significant elevation noted in ES patients than control group. Elevated hsCRP, Ferritin, Ceruloplasmin, IL-2, IL-6 is associated with ASD Eisenmenger syndrome. hsCRP and IL-2 value increase as the age of the Eisenmenger patient advances. Other inflammatory markers are evaluated in this study has no age relation. Inflammation may have some pathophysiologial role in eisenmenger syndrome like other cardiovascular disease we most often come across, like atherosclerotic heart disease, idiopathic pulmonary hypertension, rheumatic heart disease, Vasculitis. The causes, con-sequences, and prognostic significance of inflammatory markers in ES need further evaluation.

REFERENCES:


4. Reference


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<tr>
<th>Mean age (years)</th>
<th>28 ± 16.98</th>
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<tbody>
<tr>
<td>Gender</td>
<td>Male- 11 (52.38%) Female- 10 (47.62%)</td>
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<tr>
<td>WHO class n (%)</td>
<td>2: 10 (47.6%), 3: 9 (42.8%), 4: 2 (9.5%)</td>
</tr>
<tr>
<td>Mean baseline SaO2</td>
<td>88.6 ± 4.8% (72-94%)</td>
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<tr>
<td>Mean Hb (gm/dl)</td>
<td>15.1 ± 2.0 (12-20)</td>
</tr>
<tr>
<td>Cardiac defects</td>
<td>Atrial septal defect: 5 (23.8%), Ventricular septal defects: 7 (33.3%), Patent ductus arteriosus: 10 (23.8%), Complete AVSD: 3 (14.2%)</td>
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</table>

**Table 2: Inflammatory markers (mean) in Eisenmenger syndrome patients compared with those in control subjects**

<table>
<thead>
<tr>
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<th>Case(n=21)</th>
<th>Control(n=20)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Hb%</td>
<td>21± 2.88</td>
<td>19.56± 2.84</td>
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<tr>
<td>hsCRP(mg/dl)</td>
<td>1.02±2.54</td>
<td>0.71±1.26</td>
<td>0.1709</td>
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<tr>
<td>Ceruloplasmin</td>
<td>21.9±19.42</td>
<td>21.44± 11.72</td>
<td>0.2075</td>
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<tr>
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<td>438±914</td>
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</tr>
<tr>
<td>IL-2(pg/ml)</td>
<td>40.74±27.52</td>
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</tr>
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<td>3.161e-05</td>
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