Plasma Asymmetric Dimethylarginine (ADMA) levels in Children with Sickle Cell Disease and its Correlation to Pulmonary Hypertension

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Abstract

**Background:** Pulmonary hypertension (PH) is an increasingly recognized life threatening complication in Sickle cell disease (SCD), with prevalence of 10-30% in patients with associated high mortality. The etiology and pathophysiologic mechanisms are still not well understood.

**Aim of the study:** To assess the plasma levels of asymmetric dimethyl arginine (ADMA) in children with SCD and its correlation to pulmonary hypertension. **Subjects & Methods:** This study was carried out on a cohort of patients (30) with SCD followed in the Out Patients Clinic, Pediatric Department at Tanta University Hospital and 30 healthy children as a control group. Both the SCD patients and healthy control subjects had the following investigations: Blood Sampling for CBC, LDH, ferritin, reticuloctic count, bilirubin, AST, ALT, and plasma levels of ADMA were done for both groups by a commercial ADMA ELISA Kit (Immundiagnostik AG, Stubenwald-Allee 8a, D 64625 Bensheim, Germany). Doppler echocardiography was done for SCD group, SCD patients with tricuspid regurgitant jet velocity (TJV ≥ 2.5m/sec) were defined as pulmonary hypertension. **Results:** The prevalence of pulmonary hypertension was 30 % in studied patient group. ADMA plasma levels was significantly higher in patient group in comparison to the control group (p< 0.001), with a mean value 0.79 ± 0.15µmol/l, 0.46 ± 0.11µmol/l respectively and more importantly higher in pulmonary hypertension subgroup than non pulmonary hypertension subgroup (p< 0.001), with a mean value 1.10±0.11µmol/l, 0.80± 0.06 µmol/l respectively. There was a significant positive correlation between ADMA plasma levels and pulmonary hypertension (TJV ≥ 2.5m/sec) r=0.475. **Conclusion:** High plasma ADMA levels may be implicated in the pathogenesis of PH in children with SCD.

**Keywords:** Sickle cell disease, pulmonary hypertension, asymmetric dimethyl arginine.
Introduction:

Sickle cell disease (SCD) is a disease of endothelial dysfunction as it is a hemoglobinopathy that triggers erythrocyte polymerization. Increased expression of adhesion molecules on erythrocytes and endothelial cells, interactions with leukocytes, increased levels of circulating inflammatory cytokines, enhanced micro vascular thrombosis, and endothelial damage are all thought to contribute to obstruction of the arterioles by sickled erythrocytes (1). Endothelial dysfunction, defined as alterations in the normal properties of the endothelium that are inappropriate for preservation of organ function, is characterized by loss of protective endothelial characteristics. Endothelial dysfunction is characterized by decreased production and/or bioavailability of nitric oxide (NO) (2). Among the mechanisms for impaired NO synthesis is the accumulation of the endogenous nitric-oxide synthase inhibitor asymmetric dimethylarginine (ADMA) (3).

Asymmetric dimethylarginine (ADMA) is a naturally occurring amino acid. Methylated arginine derivatives were first isolated from human urine in 1970 (4). In 1992, Vallance et al (5) first described asymmetric dimethyl arginine (ADMA) as an endogenous inhibitor of the arginine-nitric oxide (NO) pathway. From then, the role of this molecule in the regulation of endothelial NO synthesis has increasingly attracted attention (6). They are synthesized during the methylation of protein arginine residues by S-adenosylmethionine:protein arginine methyl transferases (protein methylases ,PRMT). These enzymes transfer the methyl group from S-adenosylmethionine (SAM) to arginine thus forming methylated arginine and S-adenosylhomocysteine (SAH); the latter is subsequently hydrolyzed to homocysteine (7). Upon proteolytic cleavage of arginine-methylated proteins, free monomethylarginine (MMA), ADMA, or symmetric dimethylarginine (SDMA) is generated and released into the cytoplasm. MMA and ADMA, but not SDMA, act as endogenous nitric oxide synthase (NOS) inhibitors (8). Since only minor amounts of MMA are found in human plasma and SDMA has no direct effect on NOS activity, ADMA is now thought to be a major type of endogenously generated methylated arginine (2). ADMA is hydrolyzed by dimethylarginine dimethyl aminohydrolase (DDAH) to dimethylamine and citrulline (9). Pulmonary hypertension (PHT) is a recognized complication of sickle-cell disease (SCD) occurring in approximately 30% of
adult patients with sickle-cell anemia. Once manifest, it is associated with an increased risk of early death (10).

**Patient and Methods:**

*Enrollment of patient:

This study was conducted between April 2011 and April 2012 at Hematology and Oncology Unit of Pediatric Department, Tanta University Hospital. This prospective, randomized controlled study was carried out on 30 children with sickle cell disease (confirmed by hemoglobin electrophoresis) and 30 healthy children as a control group matched in ethnic, age and sex distribution. All the children in both groups were subjected to complete history taking, full clinical examination. The following data were taken from the medical records: HB electrophoresis analysis, Chest X ray, X ray on head of femur and abdominal ultrasound.

*Laboratory determinations:

Venous blood was drawn for Hemoglobin levels, leukocyte and platelet counts, reticulocyte counts, lactate dehydrogenase (LDH), Ferritin, alanine aminotransferase, aspartate aminotransferase, bilirubin, blood urea and serum creatinine were assessed in all patients, using standard methodologies. Human plasma ADMA was analyzed using a commercial ADMA ELISA Kit (Immundiagnostik AG, Stubenwald-Allee 8a, D 64625 Bensheim made in German) citrate anti-coagulated plasma of 30 sickle cell patients and 30 healthy control children were collected in complete aseptic measure. Venous fasting blood is suited for this test system centrifuged at least for 5 min at 10000 x g with in 30 min of collection. Samples were frozen at −20°C up to the measurement by ELISA.

Doppler echocardiographraphy with assessment of pulmonary hypertension using Ultrasound Machine, vivid 7, G.E was performed at Tanta university hospital, pediatric department, Pediatric Echocardiographic Laboratory according to a standardized protocol following the guidelines of the American Society of Echocardiography. Subjects were screened for PH by echocardiography performed at steady-state, defined as: ≥2 weeks from an acute illness including pain crisis, acute chest syndrome, febrile illness, or hospital admission. TRV was measured by pulsed-wave and continuous-wave Doppler
echocardiography where applicable. Multiple views (apical 4-chamber, parasternal short axis, parasternal long axis) were obtained to record optimal tricuspid Doppler flow signals, and a minimum of 5 sequential signals were recorded. The right ventricular to right atrial systolic pressure gradient was calculated using the modified Bernoulli equation \(4 \times V^2\). Pulmonary artery systolic pressure was quantified by adding the Bernoulli-derived right ventricular systolic peak pressure to the estimated mean right atrial pressure (5 mm Hg) i.e. (Pulmonary artery systolic pressure = 4\(V^2\) + right atrial pressure). Pulmonary artery diastolic pressure was estimated by measurement of the end diastolic velocity of the pulmonary insufficiency jet by similar Doppler techniques (11).

Pulmonary hypertension was defined as a peak TRV of at least 2.5 m/second equating to a pulmonary artery pressure of at least 25 mm Hg. Patients with no measurable TRV or TRV < 2.5 m/second were considered to have normal pulmonary artery pressure (12). Patients with pulmonary stenosis or other structural obstruction to pulmonary blood flow, evidence of left ventricular failure (defined as fraction shortening below 28% and ejection fraction below 50%) , atrial fibrillation or ventricular tachycardia, significant mitral valve regurgitation >2/4 or mitral valve stenosis; and severe pericardial effusion were excluded.

**Statistics:**

The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 17. The number and percent distribution of data was calculated. Chi square was used as a test of significance and when found inappropriate Fisher exact test was used. ANOVA test was used for comparison among different times in the same group in quantitative data. Correlation between variables was evaluated using Pearson’s correlation coefficient. Significance was adopted at p<0.05 for interpretation of results of tests of significance.
## Results:

Patients characteristics, clinical and laboratory data are shown in (tables 1, 2, 3). The prevalence of PH (TRV ≥ 2.5 m/sc) in studied cases is 30% (table 4). Plasma ADMA levels are significantly higher in patient than control group (table 3) with a significant higher ADMA levels in patients with pulmonary hypertension than in patients without pulmonary hypertension (table 4). LDH, reticulocytic count, serum ferritin and bilirubin levels are significantly higher in subgroup with pulmonary hypertension when compared to subgroup without pulmonary hypertension (table 5). There is a significant positive correlation between the plasma level of ADMA and LDH, reticulocytic count, ferritin and bilirubin, while there is negative correlation with HB percentage (figure 2). There is a significant positive correlation between the plasma levels of ADMA and pulmonary hypertension (figure 2).

(Tables 1-5) (Figure 2)

## Discussion:

In the current study the plasma ADMA levels was significantly higher in patient than in control group with a mean value 0.79 ± 0.15µmol, 0.46 ± 0.11µmol respectively. The same results were found in Schnog et al. 2005 (13), Kato et al. 2009 (14), and Landburg et al. 2010 (15). Elevated plasma ADMA levels lead to a reduced NO bioavailability. Potential causes of elevated ADMA concentrations occur in SCD as explained in figure (1). First due to increased release of free ADMA by increased proteolysis associated with the increased erythrocyte turnover (13) which turn over at a rate up to twenty times normal (15). Furthermore, SCD is characterized by chronically elevated vascular wall shear stress (16) Which is known to induce expression of endothelial type-I protein arginine methyltransferase, a catalyst of arginine methylation (17). In addition Billecke et al.,
2006 (18) reported for the first time the presence of a large store of protein-incorporated ADMA in close proximity to the vascular endothelium. This store may be released under certain pathological conditions. These findings may have clinical implications for those diseases displaying increased red blood cell (RBC) lysis and/or blood protein turnover. Davids et al., 2012 (19) also concluded that intact erythrocytes play an important role in storage of ADMA, whereas upon erythrocyte lysis large amounts of free ADMA are generated by proteolysis of methylated proteins, which may affect plasma levels in hemolysis-associated diseases. Second cause occurs due to impaired metabolism of ADMA following inhibition of the (DDAH), by oxidative stress triggered by several cardiovascular risk factors (20),(21). Also hypoxia and elevated levels of pro-inflammatory cytokines may inhibit or down-regulate DDAH (22). Decreased DDAH expression/activity is evident in disease states associated with endothelial dysfunction. (23). It is notably that normal arginine metabolism is impaired in SCD through the loss of de novo arginine synthesis from citrulline which occurs primarily in the kidney. Renal dysfunction, a common occurrence in SCD, will impair the major route for endogenous arginine biosynthesis (24). Plasma arginase activity is elevated in SCD as a consequence of inflammation, liver dysfunction and, most significantly, by the release of erythrocyte arginase during intravascular hemolysis, which has been demonstrated by the strong correlation between plasma arginase levels and cell-free hemoglobin levels and other markers of increased hemolytic rate (25). Low arginine bioavailability may be exacerbated further by the presence of elevated ADMA, which is a competitive inhibitor of arginine transport and all nitric oxide synthase isoenzymes (26). The state of activation or inhibition of NOS will depend on the local intracellular concentrations of substrate and inhibitor, or the L-arginine: ADMA concentration ratio (27). Thus, even modest reductions in plasma arginine concentration can significantly impact cellular arginine uptake and bioavailability (25).

(Figure 1)

On the other hand other diseases without hemolysis can induce ADMA elevation such as idiopathic PH (28), chronic renal failure and moderately increased in patients with many
other diseases including hyperlipidemia, diabetes mellitus, arterial hypertension, hyperhomocysteinemia and heart failure (7).

Our results show that the prevalence of pulmonary hypertension (TJV ≥ 2.5m/sec) represents 30% of SCD children. TJV ≥ 2.5m/sec {based on high risk of death using this value in a prospective cohort study (29)} a threshold that correlates in right heart catheterization studies to a pulmonary artery systolic pressure of at least 30 mmHg. Even though this threshold represents quite mild pulmonary hypertension, SCD patients with TJV above this threshold have a 9 to 10 fold higher risk for early mortality than those with a lower TJV. It appears that the baseline compromised oxygen delivery and co-morbid organ dysfunction of SCD diminishes the physiological reserve to tolerate even modest pulmonary arterial pressures (30). This is similar to prevalence in previous pediatric studies (31) (32) (33), (34). Also Akinsheye et al., 2010 (35) found the prevalence 30-40% of their patients with sickle cell anemia, which was associated with increased mortality. Voskaridou et al., 2007 (36) found the prevalence of PH in his study was 33%. On the other hand, Qureshi et al., 2006 (37) reported only 16% prevalence of PH in SCD; however these studies were retrospective and only a subset of the eligible patients were included. Minniti C et al., 2009 (38) showed the prevalence of elevated tricuspid jet velocity was 11%. Which is lower than the prevalence in most of the previous studies of pediatric sickle cell disease patients (31). This could be due to using a definition of 2.6m/sec or more in their studies.

This study demonstrates that age and HB percentage are not significantly risk factors for pulmonary hypertension, while reticulocyte count, LDH, ferritin and bilirubin levels are significantly risk factor for pulmonary hypertension. No correlation between age and elevated TJVs. A similar observation was made by Qureshi et al., 2006 (37) and Pashankar et al., 2008 (31).

Pulmonary hypertension can be linked to the intensity of hemolysis as serum LDH, high bilirubin levels and low total hemoglobin percentage are linked to the prevalence and severity of pulmonary hypertension (39),(40). Minniti et al., 2009 (38) generated “hemolytic index” by principal component analysis of the levels of lactate dehydrogenase, aspartate aminotransferase, bilirubin and reticulocyte count but not
hemoglobin level to achieve statistical significance, they found correlataion with elevated jet velocity. Hemoglobin concentration, a potential inverse marker of both hemolysis and hypoxia, was lower. This observation is consistent with the view that a hemolytic vasculopathy contributes to pulmonary hypertension in sickle cell disease (41). Onyekwere et al., 2008 (42) found that pulmonary hypertension was correlated with the degree of hemolysis as manifested by significantly higher lactate dehydrogenase and bilirubin, lower hemoglobin and hematocrit levels. However, after statistical adjustment for age and sex, increased serum LDH was not associated with the development of PH. Voskaridou et al., 2007 (36) found that patients with pulmonary hypertension had elevated values of reticulocyte counts and serum ferritin compared with patients without pulmonary hypertension. This consistent with Ambrusko et al., 2006 (33) who found that elevated TJV is associated with low hemoglobin, elevated reticulocyte count. Also Gladwin et al., 2004 (11) reported that in their screening study at NIH (National Institutes of Health) for pulmonary hypertension, all markers of hemolytic anemia, including low hemoglobin and hematocrit, high lactate dehydrogenase (LDH), and high aspartate aminotransferase, but not alanine aminotransferase levels, were associated with elevated pulmonary pressures. In contrast, Pashankar et al., 2008 (31), Suell et al., 2005 (34) and Ataga et al., 2004 (43) did not find a significant association between markers of hemolysis and elevated pulmonary artery pressures.

This study demonstrates that ADMA is significantly higher in pulmonary hypertension subgroup than non pulmonary hypertension subgroup. This consistent with Kato et al., 2009 who noticed that ADMA levels in patients with SCD are linked to pulmonary hypertension (44). A similar observation was found by Landburg et al., 2010 (15) who reported that plasma ADMA concentrations were significantly higher in patients with PH. Moreover, plasma ADMA concentrations were identified as a risk factor for early death in SCD. Also Landburg et al., 2008 (16) identified an association of plasma ADMA concentrations to PH in SCD, possibly identifying a novel factor of importance in its pathophysiology. They hypothesize that chronic hemolysis induced ADMA elevations significantly contribute to endothelial activation and dysfunction in SCD via NOS inhibition and that patients with higher ADMA concentrations are more prone to develop a vasculopathy leading to complications such as PH over time.
Although difference in ADMA between patients with and without PHT seems modest, even small increases in extra-cellular ADMA lead to significant intra-cellular NOS inhibition through preferred cellular ADMA uptake over arginine (45).

The present study has some limitations that deserve mention. First, one is small number of patients. Second, as regard the methodology for measurement of ADMA, in our study by ELISA. Yokoro et al., 2012 reported that ADMA has been measured by HPLC, MS however these methods have disadvantages such as difficulty in getting reproducible results and the necessity of using expensive instrument. Alternatively the ELISA method, characterized by simplicity, sensitivity and specificity can measure the concentrations of ADMA in large numbers of samples efficiently (46). Also Tsikas, 2008 concluded that an ELISA method for ADMA has become commercially available and is increasingly used in clinical studies. Comparative studies revealed that ELISA overestimates ADMA concentration as compared with LC-MS/MS. And it runs varyingly in different laboratories (47). So, Pecchini et al., 2012 have showed in their study, that appropriate calibration is needed when ADMA is measured by ELISA (48). Third, echocardiography was used to estimate the pulmonary artery pressure instead of performing a right heart catheterization to avoid subjecting the patients to invasive measures (49). Gladwin et al., 2004 (11) demonstrated a TRV ≥ 2.5 m/s to be strongly indicative of PHT in SCD, and screening for SCD-related PHT with echocardiography is now generally recommended. However, given the reported correlation between pulmonary artery pressure and the TJV in SCD, (11) it is unlikely that the lack of right-heart catheterization in the study would significantly alter the results (9).

**Conclusion:**

From this study, it can be concluded that, pulmonary hypertension is a real complication in pediatric SCD. ADMA may be implicated in the pathogenesis of SCD; and high plasma ADMA levels is a risk factor for pulmonary hypertension in children with SCD and correlated with the hemolytic markers.
**Recommendation**

Further pediatric studies are required to prove that ADMA is a novel risk factor for pulmonary hypertension in SCD.

**Funding**
The authors declare no competing financial interests.
Reference:


role of primary importance in the aetiology of sickle cell disease associated pulmonary hypertension. Chest 2008; 133:646-652.


Table (1): Demographics of studied groups.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Patients (n=30)</th>
<th>Controls (n=30)</th>
<th>P .value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-18</td>
<td>11.63±3.96</td>
<td>6-18</td>
<td>12.20 ±4.05</td>
</tr>
<tr>
<td>Sex</td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Male: female</td>
<td>16:14</td>
<td>53%: 47%</td>
<td>17:13</td>
</tr>
</tbody>
</table>
Table (2): Clinical characteristics of patient group.

<table>
<thead>
<tr>
<th>Medical history</th>
<th>Patients (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Age of onset (months)</td>
<td>8 – 36</td>
</tr>
<tr>
<td>Interval between blood transfusion (weeks)</td>
<td>2 – 7</td>
</tr>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Presentation at onset:</td>
<td></td>
</tr>
<tr>
<td>Hemolysis</td>
<td>22/30</td>
</tr>
<tr>
<td>Dactylitis</td>
<td>8/30</td>
</tr>
<tr>
<td>Cholelithiasis</td>
<td>3/30</td>
</tr>
<tr>
<td>Acute chest syndrome</td>
<td>1/30</td>
</tr>
<tr>
<td>A vascular necrosis</td>
<td>1/30</td>
</tr>
<tr>
<td>Stroke</td>
<td>1/30</td>
</tr>
<tr>
<td>Crises ≥ 2/year</td>
<td>8/30</td>
</tr>
</tbody>
</table>

ACS: acute chest syndrome, AVN: a vascular necrosis of femur.
Table (3): Laboratory data of studied groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients (n=30)</th>
<th>Controls (n=30)</th>
<th>( P ).value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB (gm/dl)</td>
<td>7.5 - 10</td>
<td>11 - 13</td>
<td>12 +1.36</td>
</tr>
<tr>
<td>MCV(fl)</td>
<td>60 - 97.7</td>
<td>74 - 88</td>
<td>80.1±3.5</td>
</tr>
<tr>
<td>MCH(pg)</td>
<td>20 - 34</td>
<td>26 - 34</td>
<td>29.8±2.3</td>
</tr>
<tr>
<td>Reticulocyte (%)</td>
<td>3 - 14</td>
<td>0.8 - 2</td>
<td>1 +1.99</td>
</tr>
<tr>
<td>White blood cell counts (thousands/µl)</td>
<td>11.68 - 22</td>
<td>4 - 12</td>
<td>7.036±1.85</td>
</tr>
<tr>
<td>Platelet counts (thousands/µl)</td>
<td>150-545</td>
<td>100 - 408</td>
<td>355.5±68.9</td>
</tr>
<tr>
<td>Lactic dehydroganase (u/l)</td>
<td>270 - 640</td>
<td>157 - 215</td>
<td>185±58.5</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>1700 - 5318</td>
<td>100 - 150</td>
<td>122.6±5.4</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>1.5 - 3.5</td>
<td>0.3 - 0.7</td>
<td>0.5 ± 0.02</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/L)</td>
<td>9 - 92</td>
<td>10 - 35</td>
<td>22.4±7.53</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/L)</td>
<td>5-80</td>
<td>7-29</td>
<td>17.2±2.6</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.3-1</td>
<td>0.3-0.9</td>
<td>0.60±0.14</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>10-82</td>
<td>15-45</td>
<td>28.4±8.2</td>
</tr>
<tr>
<td>ADMA (µmol/l)</td>
<td>0.58-1.23</td>
<td>0.33-0.70</td>
<td>0.46+0.11</td>
</tr>
</tbody>
</table>

*Values in range and mean ± SD
Table (4): Comparison of plasma ADMA level in pulmonary hypertension and non pulmonary hypertension subgroups.

<table>
<thead>
<tr>
<th>ADMA (µmol/l)</th>
<th>Tricuspid regurgitant jet velocity (TJV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TJV &lt; 2.5 m/sec</td>
</tr>
<tr>
<td></td>
<td>N=21/30 (70%)</td>
</tr>
<tr>
<td>Mean</td>
<td>0.80</td>
</tr>
<tr>
<td>±SD</td>
<td>0.06</td>
</tr>
<tr>
<td>t. test</td>
<td>5.369</td>
</tr>
<tr>
<td>P. value</td>
<td>0.001*</td>
</tr>
</tbody>
</table>
Table (5): Risk factors for pulmonary hypertension in patients group.

<table>
<thead>
<tr>
<th></th>
<th>pulmonary hypertension TJV ≥ 2.5 m/sec (n=9)</th>
<th>Non pulmonary hypertension TJV&lt;2.5 m/sec (n=21)</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>12.5 ± 1.15</td>
<td>11.2 ± 0.91</td>
<td>0.414</td>
</tr>
<tr>
<td>HB (gm/dl)</td>
<td>7.42 ± 0.30</td>
<td>7.54 ± 0.22</td>
<td>0.667</td>
</tr>
<tr>
<td>LDH (u/l)</td>
<td>935.7 ± 83.79</td>
<td>406.3 ± 45.19</td>
<td>0.001*</td>
</tr>
<tr>
<td>RETICS (%)</td>
<td>12.9 ± 0.751</td>
<td>7.7 ± 0.735</td>
<td>0.001*</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>3749 ± 307.4</td>
<td>1521.4 ± 273.77</td>
<td>0.001*</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>2.48 ± 0.185</td>
<td>1.79 ± 0.133</td>
<td>0.008*</td>
</tr>
<tr>
<td>ADMA (µmol/l)</td>
<td>1.10 ± 0.11</td>
<td>0.80 ± 0.06</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Values in range and mean ± SD
A

B

ADMA (µmol/l)

LDH (u/l)

HB %

r.0.352
p0.021*

r.0.749
p0.001
C

Ferritin (ng/ml)

ADMA (µmol/l)

r = 0.707
p = 0.001

D

Reticulocytic %

ADMA (µmol/l)

r = 0.615
p = 0.001
Figure (2): Correlations between ADMA levels, laboratory data and TJV. A: ADMA & Hb; B: ADMA & LDH; C: ADMA & ferritin; D: ADMA & reticulocyte, E: ADMA & bilirubin; F: ADMA & TJV. The reference cutoff value for ADMA levels is 0.58 μmol/l.
Figure (1): Potential mechanisms for accumulation of ADMA in SCD.

Abbreviations: ADMA, Asymmetric dimethylarginine; DDAH, Dimethylarginine dimethylaminohydrolase enzyme; NOS, Nitric oxide synthase enzyme; PRMT-1, Protein arginin methyl transferases enzyme type1; WBCs, white blood cells.