بسم الله الرحمن الرحيم
Fas and Fas Ligand in young patients with sickle cell disease

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Sickle Cell Disease

- Is a multisystem disease associated with episodes of acute illness and progressive organ damage.
- One of the most common monogenic disorders worldwide, affecting an estimated 30 million people.
SCD is a hereditary hemoglobinopathy characterized by microvascular vaso-occlusion with erythrocytes containing polymerized sickle (S) hemoglobin, erythrocyte hemolysis, vasculopathy, and both acute and chronic multiorgan injury.

SCD represents a major public health problem because of its associated morbidity and mortality.
Sickle cell disease (SCD) is a class of hemoglobinopathy, which results from a single mutation in the b-globin chain inducing the substitution of valine for glutamic acid at the sixth amino acid position.

This mutation leads to the production of abnormal hemoglobin (hemoglobin S [HbS]). In addition to homozygous SCD (HbSS), other forms such as HbSC and HbSb-thalassemia also exist.

Sickle cell anemia (SCA) is expressed as chronic hemolytic anemia and a large variety of vaso-occlusive phenomena and their consequences proliferative vasculopathy, and a predisposition to infections leading to very high early morbidity and mortality rates.
Vascular dysfunction in SCD

- Increased expression of adhesion molecules on erythrocytes and endothelial cells.

- Interactions with leukocytes

- Increased levels of circulating inflammatory cytokines

- Enhanced microvascular thrombosis
Clinical features and complications of sickle cell disease

Cerebral Vascular Bleeding (Stroke)
- Gnanopathy (Gnasher teeth)
- Auditory Impairment
- Hepatomegaly (Cholelithiasis, jaundice)
- Growth Impairment (Endocrine dysfunction)

Renal Pathology
- e.g. Haematuria, Enuresis, Papillary necrosis, Sequestration

Micro-Vascular Occlusions
- e.g. Mesenteric

Hand Foot Syndrome (Dactylitis)
- Priapism (involuntary erection of penis)
- Physical Disability (Bony deformity)

Immuo-suppression
- Chronic haemolytic anaemia
- Psychosocial implications

Diploe Expansion (Skull bone expansion)
- Retinopathy
- Cardiomegaly
- Chest Syndrome (Pulmonary)
- Splenomegaly
- Delayed Puberty
- Reduced Fertility
- Skeleto-pathology (Aplastic crisis) (Osteonecrosis) (Leg ulcers)

Obstetric Complications
Patients with sickle cell disease exhibit numerous kidney structural and functional abnormalities, changes that are seen along the entire length of the nephron.

Changes are most marked in patients with homozygous sickle cell anemia, but are also seen in those with compound heterozygous states and the sickle cell trait.

The renal features of sickle cell disease

- Hematuria
- Proteinuria
- Tubular disturbances
- Chronic kidney disease
Classification of renal manifestations of SCD based on described phenotypes

**Cortex: hemolysis-endothelial dysfunction phenotype**
- Hyperfiltration
- Glomerular hypertrophy
- Glomerulopathy
- Hypermetabolism
- CKD

**Medulla: viscosity-vasoocclusion phenotype**
- Hematuria
- Papillary necrosis
- Impaired concentrating ability
- Impaired potassium excretion
- Tubular acidosis
A member of the tumor necrosis factor (TNF) receptor/nerve growth factor receptor family.

Transduces an apoptotic signal by activating a cascade of interleukin-1B-converting enzyme (ICE)-like cysteine proteases (caspases).

45-kDa type I transmembrane protein that also exists in soluble forms.
A 40-kDa type II transmembrane protein that is expressed in activated T cells and natural killer cells and on inflammatory cells.

Exists in two forms—membrane-bound or secreted—and binds to a surface receptor called Fas on target cells and induce apoptosis.

It can be shed in a soluble form by the action of metalloproteinases.
The Fas–Fas ligand (FasL) system is recognized as a major pathway for the induction of apoptosis (programmed cell death) in cells (including monocytes and macrophages) and tissues.

Fas/FasL interactions may be related to augmentation of inflammatory response and cause massive migration of macrophages in vivo, indicating that Fas and Fas ligand act also as proinflammatory proteins.

Fas/FasL may be considered a new target for therapeutic intervention in renal injury.
Functions of Fas-FasL system:

- T-cell homeostasis
- Cytotoxic T-cell activity
- Tumor counter attack
- Immune privilege
Pathology associated with aberrant Fas/FasL activity

- Renal disease
- Hepatic disease
- Sjogren’s syndrome
- Myositis
- Down syndrome
- Human ageing
- Atherosclerotic lesions
- Nonalcoholic steatohepatitis
- Idiopathic pulmonary fibrosis
### Participation of Fas/FasL in renal injury

<table>
<thead>
<tr>
<th>FasL expressing cells</th>
<th>Cells sensitive to Fas apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesangial cells.</td>
<td>Mesangial cells.</td>
</tr>
<tr>
<td>Tubular epithelial cells.</td>
<td>Primed tubular epithelial cells.</td>
</tr>
<tr>
<td>Renal fibroblasts.</td>
<td>Renal fibroblasts.</td>
</tr>
<tr>
<td>Endothelial cells.</td>
<td>Primed endothelial cells.</td>
</tr>
<tr>
<td>Leukocytes: lymphocytes,</td>
<td>Leukocytes.</td>
</tr>
<tr>
<td>Monocytes/macrophages, neutrophils.</td>
<td></td>
</tr>
</tbody>
</table>

Fas/FasL interactions may be related to augmentation of inflammatory response

- Inflammation,
- endothelial dysfunction,
- accelerated atherosclerosis
- enhanced apoptosis

are features characteristic for CKD.

Moreover

CKD patients suffer from chronic inflammation, facilitating both Fas and FasL overexpression
Thus, the enhanced Fas–FasL binding, activating the extrinsic death receptor pathway, leads to increased apoptosis in the course of CKD

Fas/FasL may be considered a new target for therapeutic intervention in renal injury
Soluble Fas/FasL ratio as a marker of vasculopathy in children and adolescents with sickle cell disease

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Abstract

Objectives: Sickle cell disease (SCD) is characterized by chronic inflammation due to ischemic tissue damage, accentuated during acute complications. Fas and its ligand (FasL) are members of tumor necrosis factor receptor superfamily and a major pathway for induction of apoptosis. Fas/FasL interactions may be related to augmentation of inflammatory response. We assessed the levels of sFas and sFasL in 35 children and adolescents with SCD compared with 35 healthy controls in relation to hemolysis, iron overload, sickle vasculopathy including kidney disease.

Methods: SCD patients, in steady state and asymptomatic for pulmonary hypertension, were studied stressing on hydroxyurea therapy, serum ferritin, urinary albumin creatinine ratio (UACR), high-sensitivity C-reactive protein (hs-CRP) and sFas/sFasL levels.

Results: sFas/sFasL ratio was significantly higher in patients compared with controls. sFas/sFasL ratio was elevated in patients with pulmonary hypertension, nephropathy and those who had history of frequent
Determine levels of Fas, Fas ligand and their ratio in young patients with sickle cell disease.

Assess their relation to sickle vasculopathy including kidney disease.
This study was carried out in Pediatric Hematology Clinic, Pediatric Hospital, Ain Shams University including:

**Patients**

- 35 Patients with sickle cell disease

**Controls**

- 35 age- and sex-matched healthy subjects

- Patients with sickle cell disease as confirmed by qualitative and quantitative analysis of hemoglobin using high performance liquid chromatography (HPLC).

- Patients were in a steady state at time of sample collection.
Methods:

Detailed medical history:

- Demographic data.
- Age at onset and disease duration.
- Transfusion history.
- Therapy.
- Number of sickling crisis.
- History of splenectomy.
- History of viral hepatitis.
- Family history of anemia.

Thorough clinical examination.
Laboratory investigations:

- CBC.
- High performance liquid chromatography (HPLC).
- Liver and kidney functions.
- Markers of hemolysis (LDH – indirect bilirubin).
- Serum ferritin.
- Measuring serum levels of sFas, sFas ligand by enzyme linked immunosorbent assay (ELISA).
Radiological examination:

- Echocardiography
  - Left ventricular function
  - Pulmonary artery pressure

- Abdominal Sonar
Comparison of demographic data and laboratory variables among SCD patients and control group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=35)</th>
<th>SCD (n=35)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>9.1 ± 3.2</td>
<td>8.40 ± 3.69</td>
<td>0.399</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16 (45.7)</td>
<td>23 (65.7)</td>
<td>0.092</td>
</tr>
<tr>
<td>Female</td>
<td>19 (54.3)</td>
<td>12 (34.3)</td>
<td></td>
</tr>
<tr>
<td>Weight SDS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>0.79 (0.09-1.21)</td>
<td>-0.17 (-0.19-0.81)</td>
<td>0.005*</td>
</tr>
<tr>
<td>Height SDS</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Median (IQR)</td>
<td>0.2 (-0.43-0.94)</td>
<td>-0.719 (-1.306– -0.104)</td>
<td>0.002*</td>
</tr>
<tr>
<td>sFas (pg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>1000 (400-1400)</td>
<td>1400 (1300-2200)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>sFasL (pg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>200 (160-340)</td>
<td>130 (100-220)</td>
<td>0.022*</td>
</tr>
<tr>
<td>sFas/sFasL ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>5.19 (2.78-7)</td>
<td>9.23 (5.91-22)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>
sFas levels in sickle cell disease patients compared with healthy controls

sFasL levels in sickle cell disease patients compared with healthy controls
sFas and sFasL levels in relation to clinical characteristics of sickle cell disease patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>sFas (pg/mL) Median (IQR)</th>
<th>P-value</th>
<th>sFasL (pg/mL) Median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1400 (1200–1900)</td>
<td>0.170</td>
<td>145 (130–250)</td>
<td>0.261</td>
</tr>
<tr>
<td>Positive</td>
<td>2200 (1400–3200)</td>
<td></td>
<td>100 (100–160)</td>
<td></td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1350 (1100–1500)</td>
<td>&lt;0.001*</td>
<td>180 (130–275)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Positive</td>
<td>3000 (1900–3200)</td>
<td></td>
<td>100 (100–130)</td>
<td></td>
</tr>
<tr>
<td>Sickling crisis (≥3 attacks/year)</td>
<td></td>
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</tr>
<tr>
<td>&lt;3</td>
<td>1300 (1000–1400)</td>
<td>&lt;0.001*</td>
<td>200 (130–300)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>≥3</td>
<td>2800 (2050–3200)</td>
<td></td>
<td>100 (100–100)</td>
<td></td>
</tr>
<tr>
<td>Nephropathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1400 (1000–1450)</td>
<td>0.031*</td>
<td>190 (145–305)</td>
<td>0.011*</td>
</tr>
<tr>
<td>Positive</td>
<td>1900 (1300–2600)</td>
<td></td>
<td>130 (100–160)</td>
<td></td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2500</td>
<td>1400 (1200–1500)</td>
<td>&lt;0.001*</td>
<td>160 (130–250)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>≥2500</td>
<td>3200 (3000–3200)</td>
<td></td>
<td>100 (80–100)</td>
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<tr>
<td>Hydroxyurea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>3200 (2600–3200)</td>
<td>&lt;0.001*</td>
<td>100 (100–100)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Positive</td>
<td>1350 (1200–1500)</td>
<td></td>
<td>160 (130–250)</td>
<td></td>
</tr>
<tr>
<td>Iron chelation therapy</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1350 (1150–1450)</td>
<td>0.310</td>
<td>235 (175–275)</td>
<td>0.148</td>
</tr>
<tr>
<td>Positive</td>
<td>1500 (1300–2400)</td>
<td></td>
<td>130 (100–220)</td>
<td></td>
</tr>
</tbody>
</table>
sFas/sFasL ratio in relation to SCD vasculopathy, iron overload and hydroxyurea therapy
Significant positive correlation between sFas/sFasL ratio and transfusion index among patients with sickle cell disease.

Significant positive correlation between sFas/sFasL ratio and WBCs count among patients with sickle cell disease.
Significant positive correlation between sFas/sFasL ratio and serum ferritin among patients with sickle cell disease.

Significant positive correlation between sFas/sFasL ratio and urinary albumin creatinine ratio among patients with sickle cell disease.
Receiver Operating Characteristic (ROC) curve analysis of sFas, sFasL and sFas/sFasL ratio for detection of nephropathy among SCD patients.

Receiver Operating Characteristic (ROC) curve analysis of sFas, sFasL and sFas/sFasL ratio for detection of pulmonary hypertension among SCD patients.
sFas and sFas /sFasL are significantly enhanced in SCD and may be considered markers for vascular dysfunction including renal complications in those patients.

The positive correlation between sFas/sFasL ratio and WBC count strengthens the role of inflammation and apoptosis in the pathogenesis of SCD.
Elevated sFas and sFas/sFasL levels would help in early crisis prediction and to identify patients at risk of pulmonary hypertension.

Low sFas levels and sFas/sFasL ratio in patients with hydroxyurea therapy may suggest that sFas/sFasL ratio could be a potentially valuable tool for monitoring the response to therapy in SCD patients.
sFas/sFasL has Pathophysiological importance, because they provide evidence that diminishing inflammation in general, and perhaps the levels of sFas in particular, may have a role to play in altering SCD related-vasculopathy.
Analysis of the relation between sFas/sFasL and other markers of inflammation and endothelial dysfunction among SCD patients is needed to further highlight the role of sFas and sFasL levels in the pathogenesis of SCD vasculopathy.

Further longitudinal studies are also needed to verify the practical utility of sFas/sFasL measurement and their potential to reflect the severity of the clinical course in SCD patients.

The cutoff values of sFas and sFasL levels for detection of renal and pulmonary complications in SCD should be also verified in larger prospective studies.
Thank you