بسم الله الرحمن الرحيم
Long Term Survival of Protein C Deficiency in Saudi Arabia

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16th Annual Meeting of Saudi Society of Hematology
7th Pan Arab Hematology Association
February 24-25, 2018
Jeddah, Kingdom of Saudi Arabia
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The Kingdom of Saudi Arabia is the largest country in the Arabian Peninsula.

As of 1 January 2016, the population of Saudi Arabia was estimated to be 30 million (20 million Saudi; 10 million non-Saudis).
Incidence

- Purpura fulminans associated with *congenital* (inherited) protein C deficiency occurring in 1:500,000 to 1:1,000,000 live births.
- Consanguineous marriages increase the risk of inherited diseases.
- In Saudi Arabia, the rate of consanguineous marriages vary from 40%-70%.
Genetics of protein C deficiency

- Autosomal dominant fashion
- Homozygous or compound heterozygous mutations of protein C (Purpura fulminans) inherited as autosomal recessive
  - less common
  - More severe form of the disease
  - with onset of thrombotic manifestations at birth.
- Nearly 200 pathogenic mutations of this gene have been described
- These mutations are divided into 2 types:
  - Type I: Quantitative
  - Type II: Functional
PROTEIN C Deficiency

- A life threatening disorder characterized by excessive thrombosis within blood vessels and capillaries that can result in necrosis, gangrene and amputation
- Purpura fulminant (PF) can occur 2-hours after birth and can progress rapidly
- Skin lesions of PF present as redness, purplish lesions that become necrotic due to thrombosis
- Cerebral vasculature thrombosis may develop resulting in partial or complete blindness
- Hydrocephaly and severe neurological problem develop
- Large vessels venous thrombosis can occur (e.g. renal thrombosis)
Protein C is a 62-kD, vitamin K-dependent glycoprotein synthesized in the liver.

Activated protein C (aPC) exerts its anticoagulant activity primarily through **inactivation of coagulation factors Va and VIII**, which are required for factor X activation and thrombin generation.

The catalytic activity of activated protein C (aPC) is greatly enhanced by the vitamin K-dependent cofactor protein S.
Mean Protein C level in healthy individuals

- Newborns: 40 IU/dl (30% of normal adult levels)
- 6-month-olds: 60 IU/dl (45% of normal adult levels)
- Adults: 65-135 IU/dl (100% of normal adult levels)
- Normal adult levels are reached after puberty
Laboratory diagnosis

- CBC, Diff.
- Protein C(PC) antigen and activity
- PT, PTT, INR, fibrinogen, d-dimer, FDP.
- Serum: ATIII, protein S, factor V Leiden, homocystine
- Prothrombin gene 20210A mutation
- Genetic analysis
Born 20 November 2000, baby boy full term uneventful pregnancy

Assisted labor by ventous secondary to fetal distress

Apgar score of 9/1 & 9/5

Birth weight of 2.6 kg

At birth, he was found to have multiple fluid filled bullae on the left cheek, left lumbar region, right forearm and back of the head.
He was admitted to NICU as a case of epidermolysis bullas

On the 2nd day, he developed discoloration of the scrotum with worsening of the preexisting skin lesions

The diagnosis of thrombosis due to hypercoagulation defect was suspected
Physical Examination

- Normal baby.
- No dysmorphic features.
- Multiple skin lesions: left cheek 0.5 cm and abdomen 5x7 cms
- Chest, CVS, and abdomen were normal
- Genitalia: small blackish penis and scrotum
- Eyes: evidence of hemorrhage in both eyes.
Parents are 1\textsuperscript{st} degree cousin

The first child of them

Positive family history of protein C deficiency in 1\textsuperscript{st} degree cousins
## INVESTIGATIONS

<table>
<thead>
<tr>
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<th>1st day</th>
<th></th>
<th>3rd day</th>
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<tbody>
<tr>
<td><strong>Hb</strong></td>
<td>14.5</td>
<td></td>
<td>10.7</td>
<td></td>
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<tr>
<td><strong>WBC</strong></td>
<td>23.5</td>
<td></td>
<td>16.7</td>
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<td><strong>Platelets</strong></td>
<td>144</td>
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<td>102</td>
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<tr>
<td><strong>PT</strong></td>
<td>67/11.9</td>
<td></td>
<td>15.2/11.9</td>
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<tr>
<td><strong>PTT</strong></td>
<td>180/33</td>
<td></td>
<td>49/33</td>
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<tr>
<td><strong>INR</strong></td>
<td>6.9</td>
<td></td>
<td>1.3</td>
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<tr>
<td><strong>Fibrinogen</strong></td>
<td>NA</td>
<td></td>
<td>0.8</td>
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<tr>
<td><strong>D. Dimer</strong></td>
<td>NA</td>
<td></td>
<td>&gt;3,000</td>
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<table>
<thead>
<tr>
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<th>Protein C Antigen (0.70 – 1.40)</th>
<th>Protein C Activity (0.70 – 1.40)</th>
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<tbody>
<tr>
<td><strong>Child</strong></td>
<td>0.02</td>
<td>&lt; 0.01</td>
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<tr>
<td><strong>Father</strong></td>
<td>0.67</td>
<td>0.69</td>
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<tr>
<td><strong>Mother</strong></td>
<td>0.63</td>
<td>0.66</td>
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### Investigation

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
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<tbody>
<tr>
<td>ATIII O.74</td>
<td>Normal</td>
</tr>
<tr>
<td>Protein S</td>
<td>Normal</td>
</tr>
<tr>
<td>Factor V leiden</td>
<td>Normal</td>
</tr>
<tr>
<td>Prothrombin 20210A</td>
<td>Normal</td>
</tr>
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</table>

Liver and renal function are normal
CT brain showed multiple intracranial bleeding suggestive of thrombosis

MRI showed
- Extensive bleeding bilaterally
- Bleeding at temporal, occipital and parietal lobes
- Hemorrhage in both eyes
- No evidence of thrombosis in the dual sinuses

Ultrasound abdomen: normal, no thrombosis of renal veins

Scrotal ultrasound showed hemorrhage in both testes
Hospital Course

- Admitted to NICU
- Treated with FFP 10ml/kg, 6 hourly until lesions disappeared.
- Protein C was not available
- Started on LMWH
- Scrotal discoloration reappeared so FFP was given
- Transferred to pediatric ward where Warfarin was started
- At 4 months of age, patient discharged home in good general condition, on warfarin to keep INR 2.5-4
Follow Up

- Seen by Ophthalmology who diagnosed to be blind
- MRI showed bilateral retinal detachment
- At 3 months of age, he stared to have increase in head circumference
- Neurologic exam at 6 months showed increase tone and reflexes in lower limbs, increase head size and diagnosed with hydrocephalus
- Neurosurgery advised for shunt insertion
- At 5 months of age, central venous line was inserted
Follow Up

- At 6 months of age, discoloration of scrotum developed
- At 7 months of age, discoloration of lesion on the back of the head
- Treated with protein C during the acute stage and then shifted to warfarin
- At 9 months of age, he left to Germany for further management
Management

In Germany

- V-P shunt was inserted
- Circumcision was done
- Was operated on both eyes, however surgery was unsuccessful
- Central line was inserted for Protein C infusion and the mother was taught how to give it
Follow Up

- At 1 year of age, he came back from Germany
- At 2 years of age, he had right upper arm hematoma
- He developed convulsion
- EEG showed: frontal spikes
- MRI of brain showed:
  - Hemorrhage with leukoencephalomalacia
  - Hydrocephalus with V-P shunt
- Started on Vigabatrine
- Recurrent admissions
CT Brain at 5 years

- Brain atrophy with abnormal gyration and cystic encephalomalacia of the frontal lobes bilaterally and associated bilateral microphthalmia with calcification
Homozygous Protein C Deficiency with Purpura Fulminans: Report of a New Case and a Description of a Novel Mutation.

Abu-Amero KK¹, Al-Hamed MH, AL Batniji F.

Abstract

We report here a quite rare case of severe homozygous protein C deficiency. The index case is a 9-month-old Saudi boy who was born after an uneventful pregnancy at 39 weeks. The diagnosis of epidermolysis bullosa and the appearance of scrotal hematoma raised the diagnosis of thrombosis due to protein C deficiency. The clinical presentation and extremely low level of protein C activity (< 0.01 U/ml) in the index case suggested a severe case of protein C deficiency. The father is 28 years old and the mother is 25 years old and are consanguineous. Neither had a personal or family history of thrombosis.

Genomic DNA was extracted from peripheral blood of the patient and both parents, exons of protein C were amplified by polymerase chain reaction and sequenced. Sequencing revealed the presence of a novel CCTG duplicate nucleotide (effective insertion) after nucleotide 8826 in exon 9 of the protein C gene. This insertion is found in the homozygous state in the patient and in the heterozygous state in both parents. It results in a frame-shift mutation, which introduces a stop-codon, thereby generating a prematurely truncated protein. These molecular findings agree with the presence of quantitative protein C deficiency in the index case.

PMID: 12695756 [PubMed - indexed for MEDLINE]
2nd Case

- The 3rd sister to case #1, born on 10-Nov-2006
- Full term normal delivery, birth wt of 2.7 KG
- Apgar 9 at 1 and 9 at 5 minutes
- At birth, was found to have an ecchymotic lesion on the face and left flank
- Admitted immediately to NICU, where the patient was evaluated for hypercoagulation
- Referred to hematology team who advised to continue FFP transfusion every six hours
- To start protein C infusion as soon as available
Investigations

- WBC 17.3, Neutrophils 12.8.
- Hemoglobin 20.gm/dl MCV 107, MCH-34.6,
- Platelets 99
- Total bilirubin 49, direct bilirubin<1
- Sodium 136, urea 4.6, Creatinine 68, Calcium 2.6, corrected calcium 2.5, Potassium 5.3, TSH 6.3,
- PT 11, INR 1.3, PTT 35,
- Protein C level was 10 antigen (receive FFP)
- Protein C function was 0.08 (normal 0.40 ) Repeated 0.08.
CT
Multiple intracranial bleeding spots seen in the brain in both cerebral hemispheres, very close to the anterior horn and the posterior horn of the lateral ventricles as well as in the region of corona radiate on both sides. There was no active bleeding within the ventricles or in subarachnoid space.

MRI
Multiple focal areas of high signal intensity noted in D1 weighted images located at frontal lobe bilaterally, perizygonal regions bilaterally as well as right parietal region. It also revealed evidence of coarse linear hyper densities seen involving both eye globes pushing the vitreous chamber anterior more severe on the right eye representing bilateral intraorbital bleeding components.
Follow Up

* At one month of age, she developed hematomas involving legs, extending to above knee
* Treated wish protein C till lesions improve
* Ophthalmology consultation documented bilateral retinal detachment and blindness
* At 2 months of age, she was stated on warfarin to keep INR 2.5-3.5
Follow up

- At 3 years of age, central line was inserted
- At age of 4 years, she developed leg ulcer
- Warfarin was discontinued
- Protein C was started
- At 6 years of age, she started special schooling
- At present, she is in 4th grade, doing very well
- She starts feeling little pain before ecchymoses appear and asks her mom to give her Protein C
Family history

- 20 November 2000: baby boy 1st case (17 years)
- 2001: baby girl carrier
- 13/6/2002: baby girl carrier
- 15/5/2003: abortion 3/12
- 10/11/2006: baby girl 2nd case (11 years)
- 20/2/2008: baby boy carrier
- 3/6/2013: prenatal diagnosis was done and termination done
- Refused tubal ligation, plan for contraceptive
3rd CASE

- Baby boy born 29/5/2011, 37 weeks gestation
- Elective C Section, mother had 3 previous sections
- Apgar scores 8 at 1 and 9 at 5 minutes
- Birth weight 3.99kg, length 56th cm, OFC 35 cm all at 50th percentile
- Examination: pink, no dysmorphic features, skin clear, Nasal flaring, mild retractions
- Chest X ray: mild transient tachypnea of the newborn
- Admitted to NICU treated with CPAP for few hours and discharged to ward with mother
History

- Readmitted on the 4th day of life because of hematuria.
- **Umbilical vein line** was inserted and **septic** screening was done.
- Platelets were 30,000. Platelets and FFP were given.
- Bluish discoloration of the right upper and lower limb started to appear at the end of the day.
- At the age of 6 days, he was referred to hematology because of the bluish dark lesion over his right upper and lower limbs that became swollen and necrotic.
History

- No cough, wheeze or SOB
- No Cyanosis or difficulty in feeding
- No diarrhea, constipation or jaundice
- No seizures or abnormal movements
Family History

- Parents are first degree cousins
- From the south
- This child is the 4th out of four offspring
- The other siblings are well and healthy
- No family history of similar condition
- No family history of any blood disease
Physical Examination

- Looks well, afebrile
- Vital sign stable
- Chest, CVS, CNS, were normal
- Abdominal exam: necrotic lesions on the right flank, 5x5 cm
- Skin: pictures
- Eyes were normal
Investigations on 4\textsuperscript{th} Day

- U&E were normal
- Fibrinogen 2.3
- Blood goop O\textsuperscript{+}ve, DCT \textsuperscript{–}ve
- Blood Culture, urine and others were negative
- Hb was 7 gm/dl, Blood transfusion
- Platelet 30,000, given transfusion
Investigations

- PT 13.5 secs, control 8.8 secs
- PTT 35 Secs, control 33 secs
- INR 1.2; normal 0.9-1.3
- **D- Dimer were Positive**
- ATIII: 0.88, normal
- Factor V Leiden: normal
- Protein S: 0.63 (N 0.60-1.50)
- Chromosomal analyses 46XY
## Protein C

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein C antigen</td>
<td>0.02</td>
<td>0.36</td>
<td>0.56</td>
</tr>
<tr>
<td>Protein C activity</td>
<td>0.01</td>
<td>0.48</td>
<td>0.57</td>
</tr>
</tbody>
</table>
- U/S brain: normal ventricles and no bleeding
- U/S Abdomen: normal, no renal vein thrombosis
- CT brain: normal
- MRI brain and orbits: normal
Management

- FFP started 10 ml/kg 6 hourly (NICU)
- Protein C 100 IU/kg 6 hourly
- Ophthalmology consultation: normal
- Circumcision was done at 6 months of age
- Discharged home at 2 months of age with good general condition on warfarin to keep INR 2.5-3.5
Follow up

- He had 2 attacks of GE due to salmonella food poisoning treated with hydration
- Had 3 attacks of change of skin on the chin of lower limbs treated with Protein C
- At age 1½ years, porta cath was inserted
- Seen by urology for undecided tests, no intra abdominal gonads
- Seen by dental for rehabilitation
- Seen by endocrine
Follow Up

- Warfarin to keep INR 2.5-3.5
- Many admission so started on prophylaxis twice/week at age of 18 months
- Increase to three times/day after 6 month, continue the same dose till now
- NO admission for the last 3 years
Follow Up

- Now the patient is 6 years 9 month old
- Well, healthy, no bruises, no skin lesions.
- Growth parameters within normal
- Weight: 18 kg  Length: 108 cm, both on 3rd centile
- Attained his developmental milestones normally
- Reassessed by the ophthalmologist and the eyes were normal
- This year, he went to school 1st Grade
Mutation Report

Molecular characterization of novel splice site mutation causing protein C deficiency
Al-Hamed, Mohamed H.; AlBatniji, Fatma; AlDakheel, Ghadah A.; El-Faraidi, Huda; Al-Zahrani, Azzah; Al-Abbass, Fahed; Imtiaz, Faiqa

Abstract
Congenital protein C deficiency is an inherited coagulation disorder associated with an elevated risk of venous thromboembolism. A Saudi Arabian male from a consanguineous family was admitted to neonatal intensive care unit in his first days of life because of transient tachypnea and hematuria. Laboratory investigations determined low platelet and protein C deficiency. Direct sequencing of PROC gene and RNA analysis were performed. Analysis of factor V Leiden (G1691A) and factor II (G20210A) mutations was also done. Novel homozygous splice site mutation c.796+3A>T was detected in the index case and segregation was confirmed in the family. RNA analysis revealed the pathogenicity of the mutation by skipping exon 8 of PROC gene and changing the donor splice site of the exon. Detection of the molecular cause of protein C deficiency reduces life threatening and facilitates inductive carrier testing, prenatal and preimplantation genetic diagnosis for families.

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4th Case

- 4 months old girl, preterm 33 weeks
- **Diagnosed protein C Deficiency** as she had genetic test for homozygous protein was done at Germany
- Presented to PSMMC on September 2016 coming from Alhada hospital in Taif seeking further treatment and management
- Parents are Consanguineous
- Mother 26 years old, known case of protein C deficiency heterozygous, diagnosed during obstetrical investigations, had 2 abortions, one IUFD, one neonatal death at age of 2 weeks with neonatal purpura fulminant
At PSMMC

- Cranial U/S and MRI showed no intraventricular hemorrhage.
- Ophthalmological evaluation including MRI orbit showed bilateral vitreous hemorrhage with bilateral retinal detachment.
- FFP was discontinued.
- Continued on warfarin and protein C.
- For neurological follow up and repeat MRI Later.
- Back to Taif an
Conclusion

- 4 patients with purpura fulminant due to homozygous protein C deficiency
- Early recognition and treatment prevents excess morbidity and mortality.
- The clinical presentation are Acute DIC and hemorrhagic skin necrosis
- Long survival with treatment
- Prophylactic treatment is better than PRN but expensive
- Liver transplant is a cure
- Gene therapy in the future
Looking forward to Protein C deficiency cases collection in all over the kingdom through

- SAPHO Society
- Saudi Society of Hematology
- Arab Hematology Association

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THANK YOU

Acknowledgment

- The family of my patients
- My colleagues in Pediatric hematology and laboratory
- My colleagues at KFSH Research center