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Serum apelin as a novel non-invasive marker for subclinical cardiopulmonary complications in children and adolescents with sickle cell disease

Nancy Samir Elbarbary [a,*], Eman Abdel Rahman Ismail [b], Alaa Roushdy [c], Ehab Fahmy [a]

[a] Department of Pediatrics, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[b] Department of Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[c] Department of Cardiology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

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ABSTRACT

Background: Cardiovascular involvement represents a leading cause of mortality and morbidity in sickle cell disease (SCD). Apelin is a peptide involved in the regulation of cardiovascular function.
Aim: To determine serum apelin among 40 children and adolescents with SCD compared with 40 healthy controls and assess its relation to markers of hemolysis, iron overload as well as cardiopulmonary complications.
Methods: SCD patients, in steady state and asymptomatic for heart disease, were studied stressing on hydroxyurea/chelation therapy, hematological profile, serum ferritin and apelin levels. Full echocardiographic study including assessment of biventricular systolic function and pulmonary artery pressure was done.
Results: Apelin levels were significantly lower in SCD patients compared with controls ($P < 0.001$). Cardiopulmonary complications were encountered in 30% of patients. Apelin was significantly decreased among patients with cardiopulmonary disease ($P = 0.006$) whether those at risk of pulmonary hypertension ($P = 0.018$) or patients with heart disease ($P = 0.043$). Hydroxyurea-treated patients had higher apelin levels than untreated ones ($P = 0.001$). Apelin was negatively correlated to lactate dehydrogenase, indirect bilirubin, serum ferritin, end systolic diameter, tricuspid regurgitant jet velocity, right ventricle systolic pressure, pulmonary vascular resistance and tissue Doppler imaging S wave. Apelin cutoff value of 1650 ng/L could significantly detect the presence of cardiopulmonary complications in SCD with 90.9% sensitivity and 72.4% specificity.
Conclusion: Apelin is a promising marker for screening of SCD patients at risk of cardiopulmonary disease because it is altered during the early subclinical stage of cardiac affection. A combination of apelin and echocardiography provides a reliable method to assess cardiopulmonary affection in young SCD patients.

1. Introduction

Sickle cell disease (SCD) is a hereditary hemoglobinopathy characterized by abnormal hemoglobin production, hemolytic anemia and intermittent occlusion of small vessels, leading to acute and chronic tissue ischemia, chronic organ damage, and organ dysfunction [1].

With increased longevity, cardiovascular complications are increasingly evident, with the notable development of a progressive proliferative systemic vasculopathy, pulmonary hypertension, left ventricular diastolic dysfunction [2], right ventricular dysfunction, dysrhythmia, cardiac iron load and myocardial infarction [3]. Marked abnormalities in exercise capacity have consistently been seen in SCD patients. In addition to possible cardiac filling abnormalities, suggested mechanisms for this limitation in patients studied with cardiopulmonary testing include the anemia itself, pulmonary vascular disease, peripheral vascular disease, and/or myopathy [4].

Pulmonary hypertension (PH) is a common complication in patients with SCD and is associated with an increased risk of death [5,6]. Although the pathophysiology of PH in SCD is probably multi-factorial, chronic intravascular hemolysis, with associated destruction of nitric oxide (NO) by cell-free plasma hemoglobin and reactive oxygen species, appears to play a central role [7,8].

Tatemoto and colleagues [9] identified a selective endogenous ligand, named apelin. The apelinergic system distribution over such variety of tissues has suggested that it might play relevant roles in human physiology. Indeed, apelin is involved in the regulation of cardiovascular, gastrointestinal, and immune functions, as well as in bone physiology, fluid homeostasis and cardiovascular system embryonal development [10].

Apelin is localized in vascular endothelial cells while the apelin receptor (APLNR) is localized in both endothelial and smooth muscle cells in vessels and in the heart. Apelin is regulated by hypoxia inducible factor-1α and bone morphogenetic protein receptor-2. Apelin plays a role in angiogenesis and regulates endothelial and smooth muscle cell

* Corresponding author.

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apoptosis and proliferation complementary and opposite to vascular endothelial growth factor. In the systemic circulation, apelin modulates endothelial nitric oxide synthase (eNOS) expression, induces eNOS dependent vasodilatation, counteracts angiotensin-II mediated vasoconstriction, and has positive inotropic and cardioprotective effects [11].

Patients with PH have lower levels of plasma apelin, and decreased apelin expression in pulmonary endothelial cells [12]. Therefore, apelin has been proposed as a potential biomarker for PH [11]. This study sought to determine serum levels of apelin in children and adolescents with SCD and assess its relation to markers of hemolysis, iron overload as well as cardiopulmonary complications.

2. Materials and methods

This cross sectional study included 40 patients with SCD (≤18 years); 25 males and 15 females, recruited from the regular attendants of the Pediatric Hematology Clinic, Pediatric Hospital, Ain Shams University. Forty age- and sex-matched healthy subjects were enrolled as a control group (24 males and 16 females). The median age of SCD patients was 9.5 years (range: 2.5–18 years) while that of controls was 9.3 years (range: 2.5–16 years). An informed consent was obtained from the guardian of each patient or control before participation. The procedures applied in this study were approved by the Ethical Committee of Human Experimentation of Ain Shams University, and are in accordance with the Helsinki Declaration of 1975.

All patients were diagnosed with SCD based on complete blood picture, reticulocyte count and markers of hemolysis as well as hemoglobin analysis using high performance liquid chromatography (HPLC) and confirmed by genotyping based on identification of β-globin gene mutations by polymerase chain reaction and subsequent reverse-hybridization to immobilized allele-specific biotinylated oligonucleotides probes covering the most common Mediterranean mutations [13]. Exclusion criteria were infection, chronic inflammatory condition other than SCD, renal disease unrelated to SCD, symptomatic heart disease, rheumatoid arthritis or other autoimmune diseases, diabetes mellitus, or steroid therapy. Patients were in a steady state at time of sample collection and those who had sickling crisis were excluded.

All included patients were subjected to detailed medical history and thorough clinical examination with special emphasis on disease duration, history of sickling crisis, acute chest syndrome, stroke, priapism, bone manifestations, evidence of pulmonary or cardiac disease, spleen status (for HbSβ-thalassemia patients). A painful crisis was defined as the occurrence of pain in the extremities, back, abdomen, chest, or head that lasted at least two hours, led to a clinic visit, and could not be explained except by SCD [14] while steady state was defined as a period without pain or painful crisis for at least 4 weeks [15]. The frequency of sickling crisis in the previous year was divided into mild (defined as 2 or less episodes requiring medical visits) or severe (defined as 3 or more episodes requiring medical visits) [16]. The diagnosis of acute chest syndrome was defined as a new pulmonary infiltrate on chest x-ray and ≥2 of the following: chest, upper abdominal, or rib pain; dyspnea; fever; tachypnea; grunting; nasal flaring; or retractions [17].

All of SCD patients were transfused. The transfusion received was calculated as the transfusion index: volume of transfused packed red cells in mL per kg body weight per year (expressed as the mean value of the last three years).Thirty-three patients with SCD (82.5%) received hydroxyurea (Bristol-Meyers-Squibb, NY, USA) as an oral daily dose ranging from 10 to 25 mg/kg/day. SCD patients who needed chelation (57.5%) received deferoxamine therapy (Desferal®, DFO; Novartis Pharma AG, Basel, Switzerland), given subcutaneously in a dose that ranged from 30 to 40 mg/kg/day (5 days/week).

2.1. Sample collection

Peripheral blood samples were collected on potassium-ethylene diamine tetra-acetic acid (K2-EDTA) (1.2 mg/mL) for complete blood count (CBC) and hemoglobin analysis. For chemical analysis and enzyme linked immunosorbent assay (ELISA), clotted samples were obtained and serum was separated by centrifugation for 15 min at 1000 ×*g* then stored at −80 °C till subsequent use in ELISA.

2.2. Laboratory analysis

Laboratory investigations included CBC using Sysmex XT-1800i (Sysmex, Kobe, Japan), examination of Leishman-stained smears for differential white blood cell (WBC) count, hemoglobin analysis by high performance liquid chromatography (HPLC) using D-10 (BioRad, Marnes La Coquette, France), liver function tests, markers of hemolysis (lactate dehydrogenase [LDH] and indirect bilirubin) as well as serum ferritin on Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany). Serum ferritin level was measured at the start of the study with calculation of the mean value of the last year prior to the study in order to know the ferritin trend. Determination of serum levels of Apelin was done by enzyme linked immunosorbent assay (ELISA) using Wkea Med Supplies Corp (New York, USA).

2.3. Echocardiography

All studied patients were clinically asymptomatic for pulmonary hypertension and cardiovascular abnormalities. All patients were subjected to standard trans thoracic full echocardiographic study including 2D, M mode and Doppler analysis using phased array transducers of suitable frequency to age and body weight using PHILIPS Ie33 machine (Philips Medical Systems, Andover, MA, USA) by an experienced operator blinded to the laboratory results of the patients. Left ventricle (LV) dimensions, volumes and global systolic function were measured using M mode measurements as well as modified Simpson's rule. LV systolic dysfunction in the study group was considered present with LV fractional shortening <30% or LV ejection fraction <55% [18,19]. Right ventricle (RV) global function was measured using fractional area change (FAC), tricuspid annular plane systolic excursion (TAPSE), as well as Tei index. RV end diastolic diameter was measured from the apical 4 chamber view above the level of the tricuspid annulus in end diastole using standard technique. All LV and RV dimensions measured were indexed to body surface area to overcome the inert limitation of studying children and adolescents with wide age range. Further analysis of the LV and RV was done using color coded tissue Doppler imaging (TDI) of the lateral mitral and tricuspid annuli.

Screening for pulmonary hypertension was performed by non-invasive Doppler evaluation of the tricuspid regurgitation velocity (TRV) using the Bernoulli equation where pulmonary artery systolic pressure equals $4xTRV^2 + RAP$. A TRV ≥2.5 m/s was used as a proxy for patients at risk for PH [19,20]. Whenever detectable, the mean pulmonary artery pressure (MPAP) was measured using Bernoulli equation applied to the early diastolic pulmonary regurgitation Doppler signal. Pulmonary vascular resistance (PVR) was also measured using the equation $PVR_{echo} = 0.16 + 10 \times (TRV / TVI_{RVOT})$ where TRV is the peak tricuspid regurgitant velocity and TVI_{RVOT} is the velocity time integral of the right ventricular outflow tract [21]. Cardiac measurements were performed according to the guidelines of the American Society of echocardiography [22].

2.4. Statistical analysis

Analysis of data was done using Statistical Program for Social Science version 21 (SPSS Inc., Chicago, IL, USA). Quantitative variables were described in the form of mean and standard deviation or median and interquartile range (IQR; 75th and 25th percentiles). Qualitative variables were described as number and percent. In order to compare parametric quantitative variables between two groups, Student *t*-test was applied. For comparison of non-parametric quantitative variables between two groups, Mann–Whitney test was used. Qualitative variables were