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Endothelin 1 and endothelial dysfunction in children with idiopathic nephrotic syndrome

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Abstract *Background:* Endothelial dysfunction is the initial step for atherogenesis. Children with idiopathic nephrotic syndrome are at risk of endothelial dysfunction due to altered cholesterol metabolism which can lead to early atherosclerosis.
Methods: In this analytical study with longitudinal follow up 25 children with first episode of nephrotic syndrome (FENS) aged 1–16 years along with 25 age and gender matched healthy controls were enrolled. Endothelin 1 (ET 1) levels were measured by ELISA (Cloud Clone Corp. and assembled by USCN Inc, U.S.A). Other variables were measured using standard biochemical methods. Primary outcome measure was plasma ET 1 level in children with FENS and in controls. Secondary outcome measure was to observe the levels of ET 1 in children with FENS at 12 weeks in remission.
Results: The level of ET 1 was significantly higher ($p < 0.001$) in children with FENS as compared to controls. The level of ET 1 was significantly higher ($p = 0.006$) in SSNS at the onset of nephrotic syndrome and was significantly lower ($p = 0.04$) after 12 weeks of drug induced remission as compared to controls. SRNS children had higher levels of ET 1 than the steroid sensitive patients at onset but in was not statistically significant ($p = 0.062$). ET 1 showed significant positive correlation with total

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cholesterol ($r = 0.462$; $p = 0.001$) and negative correlation with serum albumin ($r = -0.565$; $p = 0.001$).
Conclusion: Plasma ET 1 levels are raised in children with FENS posing a risk of endothelial dysfunction, which persists at least in short term. Long term effects of raised plasma ET 1 needs to be studied.

Introduction

Endothelial dysfunction is characterised by a shift of the actions of the endothelium towards reduced vasodilation, a proinflammatory state and prothrombotic properties. In idiopathic nephrotic syndrome (INS), hyperlipidemia and raised apo-lipoproteins lead to oxidative stress in the endothelial cells.[1] Mechanisms that participate in the reduced vasodilatory responses in endothelial dysfunction include reduced nitric oxide generation and excess oxidative stress. INS is also a proinflammatory state associated with elevated levels of tumour necrosis factor α.[2] These factors may contribute to endothelial dysfunction in idiopathic nephrotic syndrome (INS) and subsequently lead to accelerated atherosclerosis.[3] There are several reports of atherosclerosis in children with idiopathic nephrotic syndrome.[4,5]

Studies in adult population suggest a stronger relationship between the elevated plasma levels of novel biochemical markers of endothelial dysfunction and atherosclerosis.[6] Many studies showed increased excretion of markers of endothelial dysfunction in patients with nephrotic syndrome. Adults who suffered from nephrotic syndrome in childhood have increased risk of atherosclerosis.[7] Long term studies in adult population indicate the association of cardiovascular diseases in nephrotic patients.[8] Studies in paediatric population have revealed that the risk factors of atherosclerosis occur in the patients having INS in various stages of the disease, which lead to the assumption that children with INS are predisposed to accelerated atherosclerosis.[7]

Endothelin 1 (ET 1) is produced by endothelial, vascular smooth muscle cells, and macrophages. It acts through G-protein-coupled ET (A) and ET receptors. There is evidence that ET 1 has an important role in the initiation and progression of cellular pathways leading to atherogenesis. In adults with proteinuria ET 1 levels are increased in urine.[9] Moreover ET 1 has been shown to promote microvascular platelet thrombus formation and therefore may contribute to acute coronary syndromes in this manner.[10] Oxidized low-density lipoprotein (LDL), one of the major participants in the atherogenic process, is a strong stimulus for ET production and secretion. The aim of this study was to evaluate the status of ET 1, in children with first episode idiopathic nephrotic syndrome (FENS).

Patients & methods

Study design and patient groups

This study was an analytical study with longitudinal follow-up conducted in a tertiary care hospital in New Delhi from October 2012 to March 2014. Study was approved by the institutional review board and written informed consent was taken from all the participants. For ET 1, considering mean of 54 pg/ml and standard deviation of 25 pg/ml in cases and mean of 29 pg/ml and standard deviation of 10 pg/ml in controls, using alpha error of 5% (two tail test) and power of 90% estimated sample of 13 cases and 13 controls were required. We decided to enrol 25 cases and equal number of age and gender matched controls attending the pediatric nephrology clinic. Controls were selected from the outpatient department coming for health certificate needed for joining swimming classes or dance classes. Children who had secondary nephrotic syndrome, signs of thromboembolic complications, bleeding diathesis, on drugs known to affect endothelial functions, pre-existing hypertension, diabetes mellitus, recent history of blood transfusion, who refused to give an informed consent were excluded from the study. Samples were collected at the time of initial diagnosis and before starting second line drugs. The patients were enrolled at the onset of the disease and were followed up till the end of the study period of one year. Guidelines by Indian Society of Pediatric Nephrology were used for the diagnosis and treatment of FENS, steroid dependent nephrotic syndrome (SDNS) and steroid resistant nephrotic syndrome (SRNS).[11] The primary outcome was to compare the levels of ET 1 in children with FENS compared to controls. Secondary outcome measure was levels of ET 1 in children with FENS at 12 weeks in remission.

Measurement of level of ET 1

ET 1 was measured at onset of disease and at 12 weeks of drug induced remission and sampling was done longitudinally. The blood sample (3 ml) was collected by venepuncture into a vacutainer tube (EDTA vial). Plasma was obtained by centrifuging the blood at 2800×g for 10 min. Plasma was stored at −80 °C until analysed. ELISA based measurement of ET 1 was done at Institute of Genomics and Integrative Biology. ET 1 kits were designed by Cloud Clone Corp. and assembled by USCN Inc., U.S.A. The kits contained pre-coated, standard 2 standard diluent 1 × 20 mL, detection reagent A 1 × 120 μL Assay, detection reagent B 1 × 120 μL Assay, TMB substrate and wash buffer (30 × concentrate). The measurement was done on Tecan Eliza machine present at the Institute of Genomics and Integrative Biology. This assay employed the competitive inhibition enzyme immunoassay technique. The intra-assay and inter-assay coefficients of variation were <10% and <12% respectively. The protein binding of ET 1 is 98%; hence due to this high protein binding, the measured values were corrected to plasma albumin.[12]