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Original Article

Hypoxia and Oxidative Stress Biomarkers in Hemodialysis Pediatric Patients with Chronic Renal Failure

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ABSTRACT

Background: Chronic renal failure (CRF) is responsible for high morbidity and mortality. Tissue injury due to hypoxia and/or free radicals is common in a variety of disease processes.

Objectives: This prospective study aimed to investigate effect of CRF and hemodialysis (HD) on hypoxia and oxidative stress biomarkers.

Methods: Forty pediatric patients with CRF on HD and 20 healthy children were recruited. Plasma hypoxia induced factor-la (HIF-la), vascular endothelial growth factor (VLGF) were measured by ELISA kits. Total antioxidant capacity (TAC). total peroxide (TPX). pyruvate and lactate by chemical colorimetric methods. Oxidative stress index (OSI) and lactate/pyruvate (L/P) ratio were calculated.

Results: TAC was significantly lower while TPX, OSI . VEGF were higher in patients with pre- and post-dialysis session than controls. Lactate, HIF-la levels were significantly higher in pre-dialysis session than controls. Before dialysis, l4'(and LIP ratio were lower than post-dialysis. In controls, LIP ratio correlated positively with TAC; HIF-le correlated positively with each of LIP ratio and TAC; VEGF correlated positively with each of TPX and OSI. In post-dialysis, IIF-la correlated negatively with each of TPX and OSI. In pre-dialysis session, **HIF-la** correlated positively with each of dialysis duration and plasma creatinine: VEGF correlated positively with each of dialysis duration, pyruvate and HIF-la, but, negatively with PO,; and, OSI correlated positively with "ГРХ, but, negatively with each of TAC and hemoglobin.

Conclusion: CRF patients to succumb considerable hypoxia with oxidative stress Hemodialysis ameliorated hypoxia but lowered antioxidants as evidenced by decreased levels of HIF-la and TAC in pre- compared to post-dialysis levels.

INTRODUCTION

Chronic kidney diseases (CKD) are conditions in which there **is** a functional loss of renal glomeruli caused by glomerular or interstitial renal diseases. It is a worldwide public health prod emt I) Ilypoxia is a state of reduced oxygen pressure below a critical threshold, which restricts the function of organs, tissues and cells[٣]. IIypoxic **changes in the kidney** may serve as an accelerant or progression factor for CKIt'). A group of transcription factors, designated hypoxia inducible transcription factors (HIFs), are specifically induced by low tissue oxygen tension and are likely to have a role in the oxygen-sensing

mechanism. HIF-1 is a heterodimeric protein consisting of an alpha subunit (with 3 isoforms - l a, -20. and -3(L), and a beta subunit (also known as the aryl hydrocarbon receptor nuclear translocator, ARNT), which is constitutively expressed[4]. In normoxia, the regulatory HIF- 1 a subunit is hydroxylated on two prolines by iron dependent prolyl-hydroxylases (PHD) to be degraded through the ubiquitin-proteasome pathway via its interaction with the von Hippel-Lindau (vHL) tumor suppressor protein. Under hypoxia, hydroxylase activities are inhibited to spare HIF- in that translocates into nucleus. There, it heterodimerizes with HIF-1 $_{f}$ and binds to the hypoxic response elements (HREs) of target gene regulatory sequences, resulting in the transcription of genes implicated in the control of metabolism and angiogenesis, as well as apoptosis and cellular stress response[']

Genes activated by HIF can be schematically classified into three functional groups.

1. Proteins participating in erythropoiesis, thereby increasing tissue oxygen delivery, e.g., erythropoietin, transferrin, transferrin receptor, here oxygenase-1.
2. Proteins that increase local oxygen delivery to tissues, e.g., inducible nitric oxide synthesis (iNOS) and vascular endothel^al growth factor (UEGF).
3. Proteins required for adaptation to cellular metabolism under conditions of low oxygen: glucose transporter-1 and most glycolytic enzymest[6]

VEGF is known to play multiple roles in normal renal physiology and renal disorders, but nevertheless it remains poorly understood. In the kidney, V EGF is expressed in the visceral epithelial cells of glomeruli, proximal and distal convoluted tubules and can induce nephrogenesis and vasculogenesis. It promotes cellular proliferation, differentiation, and survival and contributes to interstitial matrix remodeling[7].

Oxidative stress is imbalanced generation of reactive oxygen species (ROS) and counteracting antioxidants. In chronic renal failure (CRF), its causes are multifactorial. uremic toxins, interactions between circulating mononuclear cells and bioincompatible dialyzers or contaminated dialysate, and infections, can act as triggers[($)]. In addition, dialysis patients have reduced levels of vitamins C and E, selenium, and R-carotene, and they show lowered antioxidant enzyme activities; paraoxonase, catalase, superoxide dismutase, here oxygenase and glutathione peroxidase[9 I]. Although increased oxidative stress is increasingly recognized as an important metabolic event in adults with CRF[Iu2], there are few studies utilizing biomarkers of oxidative stress in children with CRF[9,F-15].

To date there is no prior study investigating oxidative stress and hypoxia biomarkers together in children with CRF on maintained hemodialysis (HD). We suggested that impaired response to tissue hypoxia and oxidative stress would be major determinants to clinical outcomes in such patients. This prospective study aimed at assessing the effect of CRF as well as maintenance HD on plasma oxidative stress markers [total anti-oxidant capacity (TAC), total peroxide (TPX) and oxidative stress index (OSI)] and standard hypoxia biomarkers (lactate, pyruvate and

lactate/pyruvate (LIP) ratio, and VEGF) as well as HIF-1et in CRF pediatric patients before and after **III)** session. HIF-1a, the key regulatory cellular hypoxia effector is investigated for the fïrst-time to our knowledge as a plasma biomarker. The relation between these markers and clinical characteristics of the patients will be also studied.

AIM OF THE WORK

This prospective study aimed to investigate effect of CRF and hemodialysis (I-i ^) on hypoxia and oxidative stress biomarkers.

PATIENTS AND METIHODS

Patients

Forty children and adolescents with CRF (25 male and 15 female) who were on maintenance I10 with age range from 6 - 18 years (mean + SD, 13.60 f 2.09 years) were voluntarily recruited to this prospective study from Pediatric Nephrology and Dialysis Unit. Assiut University Children Hospital. Assiut, Egypt, during the period from January 2010 to September 2010. At the time of the study. all the patients were on regular three 111) sessions per week; each time for 4 hours for more than 3 months with polysulfone dialyzing membranes, after creatinine clearance had fallen below 8 - 12 mL/min and/or pharmacological treatment and diet had proved inadequate to control clinical symptoms. The mean dialysis duration was 2.18 ± 1.36 years (range: 1.0 - 7.0 years). All patients were on dietary protein restriction, 1 g/Kg body weight per day. The dialysate used was a standard ionic composition and bicarbonate-based buffer (Na^i 138 m1VI/L, HCO_3- 36.6 mM/L, K 1.5 mM/L, Ca,' 1.25, Mg2 • 0.75 mM/L,CI- 107 mM/L) in all cases. The blood flow was 100 - 120 mL/min. Vascular access was arteriovenous fistula in the upper limbs. The causes of CRF were: urolithiasis (n --- 11), recurrent pyelonephritis with vesico-ureteral reflux (n 8), chronic glomerulopathies (n _ 16), hypoplastic-dysplastic kidney (n = 2), posterior urethral valve (n =- 2) and cystinosis (n 1). The healthy control group consisted of 20 age- and sex-matching volunteers aging 8 - 18 years (13.00 t 2.81 years) admitted from those coming for routine check-up.

At the time of entry, none of the participants was receiving non-steroidal anti-inflammatory drugs, immunosuppressive therapy or any other drugs that might have interfëred with the oxidative stress, and none had uncontrolled hypertension or a history of seizure. There was no evidence of iron. folic acid, or vitamin **B 2** deficiency. Albumin or blood transfusions had not been given in the 6-week period prior to investigation. Patients with infections, vasculitides, or respiratory, metabolic and hepatic diseases were excluded. Control subjects were on a normal diet without vitamin supplementation and were free from any infection or medication. Routine analyses showed that controls had normal renal function tests and no disorders of lipid-metabolism. Both patients and controls were nonsmokers. The study was approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki and informed consent was obtained in every case from their legal guardians. Demographic, clinical and

biochemical characteristics of all participants were reported.

Laboratory investigations:

Blood samples (5 mL) with and without EDTA/sodium fluoride as anticoagulant were obtained via venipuncture after the participants had fasted overnight (between 8.30 p.m. and 9.30 a.m.), and serum and plasma were immediately separated. Following centrifugation (3000 rpm for 15 minutes), samples of plasma and serum were stored at - 80"C **until.** analysis. In HI) patients, venous blood samples were drawn immediately before and **after hemodialysis** session. Baseline laboratory investigations were carried out for **all patients and controls** including complete blood count, blood **urea nitrogen, serum creatinine, pH. blood gases and infection screening, which included blood and urinary cultures by standard methods. Arterial pH, $Pa0_2$, $PaCO_2$, $HC0_3^-$ were determined using a pH/blood gas analyzer on anaerabically collected** blood drawn in ice cooled, **heparinised glass syringes.**

Specific ELISA assays were utilized to measured each of VEGF (Cat# ELH-VEGF-001, RayBiotech, Inc, Norcross GA 30092 - lower detection limit is 10 pg/mL) **and HIF-1a (Cat# DYGl935-5, R&D Systems, Minneapolis, MN 55413, USA - lower detection** limit is 3 **pg/mL) with specific capture and biotinylated detection antibodies and Streptavidin-Horse Radish Peroxidase / tetramethylbenzidine as the detection system. Total antioxidant capacity was estimated according to the colorimetric method of Koracevic et** al.[16] **with uric acid** as standard. Total plasma peroxide **concentrations were determined using the calorimetric FOX2 method with Hıo, standard as modified by Harma et a1.** [171]. **Briefly, 250 µM FOX2 reagent (9.8 mg ammonium ferrous sulfate in 10 mI. of 250 mM** HbSO4 was added to 90 mL. HPLC-grade methanol containing 79.2 mg butylated hydroxytoluene (BHT), finally, 7.6 mg xylenol orange was added. The blank reagent is the same with omission of ferrous sulfate. 200 µI., of plasma was incubated with 1.8 mL FOX2 or blank reagent at room temperature for 30 min. Tubes were centrifuged at 12,000 g for 10 min and absorbance of clear supernatant was determined at 560 rim vs. blank. The coefficient of variation for individual plasma samples was less than 5%. Percent ratio of TPX to TAC level is the oxidative stress index (OSI [(TPX, ЦМ/L) / (TAC. цMIL) X 100J)[17181]. Pyruvate was measured following the decrease in UV-absorbance of **$NADH.H^+$ according to instructions** of the manufacturer (Cain 180 000, **Greiner Diagnostics GmbH, D-79353 Bahlingen, Germany). Lactate was colorimetrically measured using lactate oxidase / Peroxidase/ tribromo-hydroxybenzoic acid-4-aminoantipyrine according to** instructions **of the** manufacturer (Cat# 274 **001, Spectrum Biodiagnostics, Cairo, Egypt). Lactate/pyruvate ratio** was calculated.

Data analysis:

Statistical Science for Social Package (SPSS **Inc, USA) software** computer program version 12 was used for data analysis. Data were presented as mean +/- standard deviation (SD) or number and percentage (n, %) as appropriate. For comparison of two groups the nonparametric test for **independent variables and paired and**

unpaired student "t" test for parametric variables were used while, comparisons of multiple groups were done using one-way analysis of variation (ANOVA) for parametric variables. Spearman's correlation test was used for parametric variables. For all tests, a probability (p) < 0.05 was considered significant.

RESULTS

Table (1) showed relevant patients' characteristics. Total antioxidant capacity was significantly lower while TPX, OSI and VEGF were significantly higher in patients with pre- and post-dialysis session than healthy controls. Lactate and HIF-la levels were significantly higher in pre-dialysis session compared to healthy controls. Before dialysis, TAC and L/P ratio were higher compared to post dialysis level (Table 2).

In healthy controls and post-dialysis, pyruvate correlated positively with lactate but negatively with L/P ratio; OSI correlated positively with TPX. In healthy controls, L/P ratio correlated positively with TAC; HIF-la correlated positively with each of L/P ratio and TAC; VEGF correlated positively with each of TPX and OSI. In post-dialysis, HIF-1 a correlated negatively with each of TPX and OSI (Table 3).

In pre-dialysis session, dialysis duration correlated positively with PCO2 but negatively with each of hemoglobin and P02. Creatinine correlated positively with each of PCO2 and HCO_3^-, but negatively with pyruvate. $P0_2$ correlated positively with TAC, but negatively with each of PCO2 and $HC0_3$. HCO3 correlated positively with pH, but negatively with TPX. LIP ratio correlated positively with each of creatinine, PCO2 and lactate, but negatively with each of $P0_2$ and pyruvate. HIF-1 a correlated positively with each of dialysis duration and creatinine. VEGF correlated positively with each of dialysis duration, pyruvate and HIF-la, but negatively with $P0_2$. Oxidative stress index correlated positively with TPX, but negatively with each of TAC and hemoglobin (Table 4).

Table 1: Relevant patients' characteristics.

Parameters	Mean ± SD	Range
Time on hemodialysis (years)	2.18 ± 1.36	1.00-7.00
Hemoglobin (gram/dL)	8.47 ± 2.12	3.70-11.60
Creatinine (μM/L)	468.00 ± 177.55	157.00-750.00
pH	7.29 ± 0.06	7.15-7.35
PCO_2 (mmHg)	31.74 ± 6.92	18.20-43.10
PO_2 (mmHg)	106.40 ± 43.64	42.00-169.00
HCO_3 (μM/L)	15.66 ± 4.13	8.10-21.20

KOH

Table 2: Changes in the plasma levels of the investigated hypoxia and oxidative stress biomarkers in renal failure patients before and after hemodialysis session and healthy controls.

Parameters	Control (n = 20)	Before dialysis (n = 40)	After dialysis (n = 40)
Age (years)	13.00 ± 2.81 6.00-18.00	13.60 ± 2.09 6.00-18.00 * p < 0.494	
Gender			
Male	10 (50.00%)	25 (62.50%)	
Female	10 (50.00%)	15 (37.50%)	
Total antioxidant capacity (mM/L)	0.66 ± 0.52 0.57-0.75	0.60 ± 0.05 0.54-0.72 *** p < 0.0001**	0.57 ± 0.06 0.47-0.69 *** p < 0.0001** p < 0.036**
Total peroxides (μM/L)	30.50 ± 12.76 10.00-40.00	38.50 ± 12.52 10.00-60.80 *** p < 0.021**	37.25 ± 12.92 10.00-50.00 *** p < 0.050** ** p < 0.854
Oxidative stress index	4.63 ± 1.89 1.56-6.35	6.52 ± 2.30 1.56-9.38 *** p < 0.002**	6.53 ± 2.23 1.75-10.64 *** p < 0.002** ** p < 0.982
Lactate (mg/dL)	12.06 ± 3.66 8.13-18.64	15.90 ± 6.56 7.63-25.48 *** p < 0.019**	13.34 ± 5.99 5.99-28.36 * p < 0.425 ** p < 0.054
Pyruvate (mg/dL)	5.02 ± 3.67 2.00-12.00	6.47 ± 8.69 1.60-42.80 * p < 0.402	6.84 ± 4.02 2.00-14.40 * p < 0.280 ** p < 0.766
Lactate/pyruvate ratio	3.36 ± 1.69 1.55-5.88	4.07 ± 2.70 0.59-8.42 * p < 0.234	2.57 ± 1.76 1.00-8.31 * p < 0.187 **** p < 0.003**
Vascular endothelial growth factor (pg/mL)	4.00 ± 1.26 2.50-5.00	32.91 ± 24.40 3.16-65.26 *** p < 0.0001**	33.15 ± 24.58 1.89-74.56 *** p < 0.0001**** p < 0.960
Hypoxia-induced factor-1α (pg/mL)	29.45 ± 10.07 24.54-49.08	57.02 ± 41.86 24.54-162.96 *** p < 0.002**	43.29 ± 26.67 24.54-100.46 * p < 0.115 ** p < 0.056

* p: significance versus healthy controls; ** p: significance versus pre-dialysis session.

Table 3: Correlations between the measured parameters in healthy controls and after dialysis patients.

Parameters	Lactate	Pyruvate	Lactate/Pyruvate ratio	Hypoxia induced factor-1α	Vascular endothelial growth factor	Total antioxidant capacity	Total Peroxides
Pyruvate							
Control	**0.777 (0.0001)**						
After dialysis	**0.533 (0.0001)**						
Lactate/pyruvate ratio							
Control	-0.287 (0.219)	**-0.817 (0.0001)**					
After dialysis	0.212 (0.188)	**-0.630 (0.0001)**					
Hypoxia induced factor-1α							
Control	0.228 (0.219)	-0.366 (0.113)	**0.767 (0.0001)**				
After dialysis	0.048 (0.770)	0.015 (0.929)	0.229 (0.156)				
Vascular endothelial growth factor							
Control	**0.489 (0.029)**	0.267 (0.256)	0.051 (0.832)	0.408 (0.074)			
After dialysis	0.125 (0.441)	0.059 (0.718)	0.098 (0.545)	-0.163 (0.314)			
Total antioxidant capacity							
Control	0.236 (0.316)	-0.370 (0.109)	**0.721 (0.0001)**	**0.940 (0.0001)**	0.415 (0.069)		
After dialysis	0.048 (0.771)	-0.148 (0.361)	0.105 (0.517)	-0.220 (0.173)	-0.147 (0.366)		
Total peroxides							
Control	0.206 (0.383)	0.029 (0.904)	0.037 (0.878)	0.382 (0.097)	**0.525 (0.017)**	0.350 (0.130)	
After dialysis	0.036 (0.825)	0.070 (0.668)	-0.204 (0.207)	**-0.529 (0.0001)**	-0.006 (0.972)	0.168 (0.301)	
Oxidative stress index							
Control	0.164 (0.489)	0.113 (0.635)	-0.124 (0.604)	0.192 (0.416)	**0.463 (0.040)**	0.146 (0.540)	**0.978 (0.0001)**
After dialysis	0.015 (0.929)	0.113 (0.489)	-0.240 (0.136)	**-0.459 (0.003)**	0.062 (0.705)	-0.155 (0.340)	**0.944 (0.0001)**

Table 4: Correlations between the measured parameters in predialysis patients.

Parameters	Dialysis duration	Hemo-globin	Creatinine	pH	PO_2	PCO_2	HCO_3	Lactate	Pyruvate	Lactate/ Pyruvate ratio	HIF-1α	VEGF	Total anti-oxidant capacity	Total Perox-ides
Hemoglobin	**-0.362 (0.022),**													
Creatinine	0.139 (0.392)	0.110 (0.501)												
pH	0.144 (0.376)	0.085 (0.602)	0.226 (0.161)											
PO_2	**-0.473 (0.002)**	0.001 (0.996)	0.163 (0.316)	0.136 (0.402)										
PCO_2	**0.360 (0.022)**	0.258 (0.108)	**0.386 (0.014)**	0.236 (0.143)	**-0.629 (0.0001)**									
HCO_3	-0.219 (0.174)	0.252 (0.116)	**0.386 (0.014)**	**0.612 (0.0001)**	**-0.435 (0.005)**	**0.911 (0.0001)**								
Lactate	-0.208 (0.197)	0.197 (0.223)	0.177 (0.273)	-0.040 (0.805)	-0.254 (0.114)	0.308 (0.053)	0.211 (0.192)							
Pyruvate	-0.193 (0.234)	0.089 (0.585)	**-0.462 (0.003)**	-0.177 (0.273)	-0.278 (0.083)	0.094 (0.562)	0.004 (0.981)	0.282 (0.078)						
Lactate/ pyruvate ratio	-0.076 (0.640)	-0.023 (0.889)	**0.589 (0.0001)**	-0.116 (0.477)	**-0.323 (0.042)**	**0.418 (0.007)**	0.263 (0.102)	**0.513 (0.001)**	**-0.457 (0.003)**					
HIF-1α	**0.677 (0.0001)**	-0.053 (0.745)	**0.342 (0.031)**	0.159 (0.328)	0.225 (0.163)	-0.112 (0.493)	-0.034 (0.837)	0.125 (0.442)	-0.149 (0.359)	0.220 (0.173)				
VEGF	**0.486 (0.001)**	0.152 (0.350)	0.021 (0.899)	0.070 (0.667)	**-0.381 (0.015)**	0.301 (0.059)	0.265 (0.098)	0.291 (0.069)	**0.316 (0.047)**	0.027 (0.866)	**0.374 (0.018)**			
Total antioxi-dant capacity	0.265 (0.098)	0.218 (0.176)	-0.174 (0.284)	0.079 (0.629)	**0.488 (0.001)**	-0.290 (0.070)	-0.185 (0.252)	-0.174 (0.283)	-0.147 (0.366)	-0.142 (0.384)	0.068 (0.676)	-0.206 (0.202)		
Total peroxides	-0.017 (0.918)	**-0.387 (0.014)**	0.190 (0.239)	-0.228 (0.158)	-0.044 (0.788)	-0.307 (0.054)	**-0.361 (0.022)**	-0.032 (0.843)	-0.194 (0.230)	0.128 (0.430)	-0.138 (0.394)	0.229 (0.156)	-0.247 (0.124)	
Oxidative stress index	-0.093 (0.566)	**-0.434 (0.005)**	0.209 (0.195)	-0.237 (0.141)	-0.165 (0.308)	-0.206 (0.202)	-0.284 (0.075)	-0.002 (0.989)	0.161 (0.322)	0.156 (0.337)	-0.172 (0.287)	0.245 (0.128)	**-0.469 (0.002)**	**0.969 (0.0001)**

VEGF, vascular endothelial growth factor; HIF-1α, hypoxia induced factor -1α

DISCUSSION

Relative hypoxia, as the major hypoxia-inducible factor activation, is detectable in chronic kidney disease tissues irrespective of etiology and is thought to result from a combination of structural and functional changes that include decreased peritubular blood flow associated with glomerular injury, capillary rarefaction, vasoconstriction, luminal narrowing of atherosclerotic vessels, increased oxygen demand from hyperfiltration and tubular hypertrophy, limited oxygen diffusion as a consequence of extracellular matrix expansion, and renal anemia [19]. Chronically ill patients on HD exist in a unique situation because their survival is dependent upon treatments which are operational only 12 hours per week in four hour sessions. This procedure subjects these patients to innumerable abrupt alterations in the internal environment, especially rapid shifts in pH [20]. In this study, the pre-dialysis levels of plasma lactate, HIF-la and VEGF were significantly higher compared to healthy controls while, lactate/pyruvate ratio was significantly higher compared to post-dialysis level. The lactate level tended to fall with dialysis, at least in part attributable to this procedure. Also, VEGF plasma level was significantly elevated in post-dialysis sessions compared to healthy controls. Hypoxia is accompanied by a significant increase in blood lactate and severe systemic acidosis as a direct effect of anaerobic metabolism. Beside the direct effects of anaerobic metabolism, catecholamine-induced stimulation of cellular glycolysis and subsequent synthesis of lactate worsens the increased systemic lactate. In such conditions, accumulated pyruvate is metabolized into lactate. Determination of the lactate/pyruvate ratio thus provides a more accurate statement of tissue metabolism and the cytosolic redox condition([11]). As a correction measure, hypoxia-induced HIF-1 also upregulates the expression of monocarboxylate transporter 4, which mediates lactic acid efflux, and of membrane-bound carbonic anhydrase IX, which catalyses the conversion of extracellular CO, to carbonic acid (H_2C0_3). The latter contributes to the acidification of the extracellular space and enables an increase in intracellular pH through the subsequent uptake of $HC0_3$ (a weak base). HIF-1 directly activates the expression of several pro-angiogenic factors, the best characterized of which is VEGF. This event promotes the formation of new blood vessels, thus restoring the supply of oxygen and nutrients[12]. Increased HIF expression has been found in animal models of CKD and in renal biopsy material from patients with diabetic nephropathy and other forms of renal diseases [13,14].

In this study, HIF-1 a was investigated as a plasma biomarker for the first-time world-wide particularly in CKD. In healthy controls HIF- I x correlated positively with LIP ratio. In pre-dialysis session, dialysis duration correlated negatively with hemoglobin, PO, but positively with PCO_2. HIF-1 a correlated positively each of dialysis duration and creatinine level. VEGF correlated positively with each of dialysis duration, pyruvate, and IF-In, but negatively with PO,. The positive correlation found in our study between plasma Ih F-1a with dialysis duration and creatinine

concentration in children treated with maintenance HD demonstrates that hypoxia highly impacts the renal functions. The positive correlation between 'IF-Ia and VEGF is expected as VEGF gene is HIF-1 a-inducible to increase local oxygen delivery to tissues. This indicates the utility of HIF-la as plasma biomarker of cellular hypoxia in CKD that reflects the disease outcomes.

The role of ROS and/or decreased antioxidant activity in the development of CRF complications, such as atherosclerosis and related cardiovascular disturbances is well-estab ished[▾ n]. In this study children with CRF showed increased plasma TPX and OSI and decreased TAC levels in both pre- and post-dialysis compared to healthy controls. Increased oxidative stress has been reported in numerous adult studies in patients with CRF[^^ 25]; however, there are few reports on the anti-oxidant system in children with CRF [2627]. Zwolinska et al.[14] reported increased lipid peroxidation, monitored by plasma and erythrocyte malondialdehyde (MDA), together with decreased superoxide dismutase, catalase and glutathione peroxidase activities in children with pre-dialysis chronic renal failure as compared to healthy age-matched subjects. Evidence has accumulated that oxidative stress is present in HD patients. The results of our study show significant decreased in TAC levels in post-dialysis compared to pre-dйalysis which may be related to the loss of antioxidants during dialysis. Turi et aI.[12] observed an increase of erythrocytic- MDA in six HD children. Daschner et a1.[12] : showed high plasma MDA levels in a group of ten pediatric patients on HD and in eleven children on peritoneal dialysis. Other studies indicate an impairment of antioxidant systems and augmentation of oxidants during HD sessions in adult patients with end stage renal diseases[13o-321]

One reason for oxidative stress in patients with renal failure is the underlying disease itself. Renal toxicity and immunological disorders of the kidney result in an elevated formation of ROS which is active in the pathogenesis of kidney disease. However, treatment procedures were also shown to induce oxidative stress [1331]. During HD, the absence of complete correction of the uremic toxicity together with the untoward effects of dialysis, malnutrition and the progressive worsening of clinical condition, can lead to oxidative stress - caused by an abnormal production of oxidants including ROS and uremic toxins with pro-oxidant function, and defective antioxidant protection. Bioincompatibility of dialysis membranes represents an important source of ROS. Losses of antioxidants via dialysis are the factors that may be responsible for the imbalance between pro-oxidative and antioxidative mechanisms in HD patients [134]. Total antioxidant capacity in renal failure group is diminished to a great extent due to antioxidant exhaustion and inhibition [13j]. Oxidative stress can also accelerate the apoptosis of leukocytes in HD patients [1361]. The activation of neutrophils and the complement pathway during HD session as the result of interactions of the blood with the dialysis membrane and endotoxin contaminated dialysate, iron overload, the presence of advanced glycation end products, high

homocystcine levels, intradialytic cytokine activation, among others, could play a rolet[37].

However, Drai et al[38] reported that chronic renal disease patients who were admitted to hemodialysis, presented enhanced levels of MDA and oxidized glutathione, and decreased concentrations of glutathione and glutathione peroxidase, without any changes in plasma levels of TAC, vitamins A and E. Another clinical study reported that TAC measured even by "Trolox equivalent antioxidant capacity" (TEAC), than ferric reducing--antioxidant power (FRAP) was higher in herodialysis patients compared with control group. Although MDA and 4-hydroxynonenal (4-HNE) levels were also increased, plasma thiols were lower and a-tocopherol was not alteredt[39]. After hemodialysis, plasma levels of thiols, MDA, 4-HNE and TAC were normalized. Similar results were found by Samouiliiou & Grapsat[40]. Difference in the biomarker used and dietary antioxidant supplementations could be a major cause of such discrepancy.

In our healthy controls, L/P ratio correlated positively TAC; HIF-1 a correlated positively with each of LIP ratio and TAC; VEGF correlated positively with each of TPX and OSI. In post-dialysis, HIF-1o correlated negatively with TPX, OSI. In pre-dialysis session, a positive correlation between P02 with TAC, but negative correlations between HCO3 and TPX, and, between OSI and hemoglobin were reported. In this respect, others[1225.27)] reported an inverse correlation between MDA serum concentration and hemoglobin in the blood of HD patients. The accelerated LPO at the low 1-lb level might be explained by oxidative stress due to the anemic condition itself. Anemic patients showed an increased frequency of ventilation at peak exercise because of the limited oxygen transport capacity, implying anaerobic metabolism due to hypoxemia and ischemia.

Conclusion: There is considerable hypoxia in pediatric patients with CRF that leads to oxidative stress. HD leads to decreased hypoxia but increased loss of antioxidants as evidenced by decreased levels of HIF-1a and TAC in pre-compared to post-dialysis levels. The resulting oxidative stress could contribute to the long-term complications in uremic patients. In compensation, VEGF levels increase to improve oxygen delivery to hypoxic tissue. Further longitudinal studies are necessary to establish the relationship between plasma markers of hypoxia and oxidative stress and clinical outcomes of CKD on maintained HD - particularly utilizing interventions such as antioxidant supplements and improved aeration.

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