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Assessment of Growth and Oxidized High-Density Lipoprotien Level in Children on Regular Hemodialysis

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Background: The cause of growth failure in chronic kidney disease (CKD) is multi-factorial with linear impairment being a final common pathway of various factors including malnutrition, increased catabolism, loss of nutrients and antioxidants, and aggressive dietary restrictions during dialysis .Anemia, metabolic acidosis and persistent micro inflammations are also causative factors.

Objectives: To assess growth in children with CKD on regular hemodialysis and measure oxidized high density lipoprotein (OX-HDL), highly sensitive C-reactive protein (hsCRP) and to discuss their roles as a risk factors of malnutrition among studied cases.

Patients and Methods: The study included 25 children with end stage renal disease (ESRD) on regular hemodialysis more than 6 months. Their ages ranged from 5-15 years with a mean of 10.76 years, also 25 of apparently healthy, age and sex matched were included. The study sample was selected from pediatric dialysis unit and outpatients clinic, of AL-Zahraa University hospital .Anthropometric measurements were assessed with determination of OX-HDL and hsCRP serum levels in both cases and the controls.

Results: Anthropometry showed significant decrease in weight, height, body mass index, mid arm circumference and triceps skin fold thickness in patients when compared to the controls. 100% of patients had high level of OX-HDL and hsCRP, and positive correlation between OX-HDL and Z-score for Wt and Ht were detected.

Conclusion: Malnutrition, and growth delay are common in children with chronic kidney disease on regular hemodialysis. High rate of inflammation in dialysis children as there were high level of hsCRP and OX-HDL. HDL, in regular hemodialysis children loses its protective function as an anti-oxidant, anti-inflammatory and becomes pro-inflammatory factor.

Key words: CKD, dialysis, OX-HDL, malnutrition

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INTRODUCTION

The growth failure in CKD children is multi-factorial, the age at onset of the disease, primary renal disease, severity of CKD, hormonal resistance, chronic anemia, metabolic acidosis, malnutrition, renal osteodystrophy, persistent micro-inflammation, recurrent infection, cardiac dysfunction and inadequate dialysis are all implicated [(1)].

Despite good progress with regard to both conservative treatment and renal replacement therapy (RRT), 30% to 60% of children with end stage renal disease (ESRD) still grow up to become stunted adults[(2)], psychosocial problems in children with CKD, the age at onset of ESRD, the duration of chronic renal failure, gender and primary disease were related to final height[(3)].

High-density lipoprotein (HDL) is known as a "good" lipoprotein, because high levels seem to protect against advancing atherosclerosis by carrying cholesterol away from the arteries and back to the liver. HDL possesses antioxidant and anti-inflammatory activities in interactions with circulating cells, inhibiting leukocyte and platelet activation and thus exerting further systemic anti-inflammatory actions [(4)].

In the presence of systemic inflammation and oxidative stress, these antioxidant enzymes can be inactivated and HDL can accumulate oxidized lipids and proteins that result in a pro-inflammatory nature. Under these conditions, the main protein of HDL, apo-lipoprotein (apo) A1, can be modified by reactive oxygen species. Alterations of

HDL by oxidation impair the capacity to promote cholesterol efflux from monocytes and macrophages and may result in a loss of anti-inflammatory effects and a resulting change to a pro-inflammatory nature [5].

Oxidized HDL (OX-HDL) as a pro-inflammatory HDL cholesterol (HDL-C) is thus thought to represent a risk factor for mortality and morbidity in MHD patients[6].

Highly sensitive C-reactive protein (hsCRP) was assayed as a marker of chronic inflammation. Recent studies have accumulated compelling evidence for a role of C reactive protein (CRP) in improving risk prediction in this setting. In the large cardiovascular health study, renal insufficiency was independently associated with elevation of CRP, which may indicate an important pathway mediating the increased cardiovascular risk in persons with kidney disease. Elevated CRP levels are associated with an increase in the carotid intima-media area in CKD-pre-dialysis and dialysis patients[7].

PATIENTS AND METHODS

Across section comparative study included 25 children with ESRD (eGFR <15 mL/min/ 1.73 m2) on regular hemodialysis more than 6 months. They were dialyzed for 4 hours/ setting, 3 times weekly; they were 13 males and 12 females, their ages ranged from 5-15 years with a mean of 10.76 years. The most common cause of ESKD group was unknown (30%) followed by reflux nephropathy (20%) and chronic nephritis(20%). The mean duration of dialysis was (1.9±1.3) years and the mean Kt/V (1.52±0.219).Also 25 of apparently healthy, age and sex matched children included as a control group. They were selected from children attending the hemodialysis unit and outpatient pediatric clinic of Al-Zahraa university hospital. Patients with acute and chronic infections, central venous catheter, chronic illness (hepatic disease, diabetes, chronic heart disease…) and children on medications as (anti-oxidant,anti-inflammatory, immunosuppressive, antibiotic and growth hormone were excluded . Informed consent was obtained from the participating patients or their parents in adherence with the guidelines of the ethical committee of AL-Zahraa university hospitals, AL-Azher University, Cairo, Egypt.

All studied children were subjected to:

1-Full history taking with detailed renal history for patients including*:*

- Duration of kidney impairment and dialysis, etiology of chronic kidney disease, dietetic history drug history and history of any other disease.
- **Complete general and local examination**
- **Anthropometric measurements** assessment was made using standardized equipment, following the recommendations of the international biological program (IBP) [8]. Measurements taken included: weight, height, body mass index, mid arm circumference and triceps skin fold thickness (TSF). Weight measures were obtained in euvolemic weight (post dialysis). Growth evaluation was based on the Egyptian Growth Reference Data[9].

Laboratory investigations

Sample collection:

5 ml venous blood samples were withdrawn under complete aseptic precautions from patients and control (before connecting the patient to the HD machine from the needle inserted in the AVF) and divided into 2 specimens

1- A specimen on EDTA tubes for complete blood picture.
2- A specimen on plain tubes for assessment of serum creatinine, urea, albumin, on the same day. After centrifugation of the samples and separation of sera. The rest of the sera samples were separated in epindorph after careful labeling and stored at -20c till the time of the assay of OX-HDL and hsCRP by ELISA kit.

Statistical Analysis

The data were collected, revised and entered to the statistical package for social science (SPSS) version 16 and the following were done: The data were presented as mean, standard deviation and range for the quantitative data and as a number and percentages for the qualitative

data, and the comparisons was done between two groups with quantitative data with normal distribution using the independent sample t-test and Mann-Whitney test if the distribution is not normal

Z score was calculated for weight (Wt), height(Ht), body mass index (BMI), mid arm circumference (MAC) and triceps skin fold thickness (TSF) according to the following formula:

$$Z=\frac{\chi - \mu}{\sigma}$$

χ is the individual patient value

$\mu$the mean value for the normal reference Egyptian population.

σ the standard deviation from normal value.

the P-value was considered significant as the following:

P > 0.05: Not significant

P < 0.05: Significant

P < 0.01: highly Significant

Results

Table 1: Showed comparison between patients and the controls regarding weight, height and BMI centiles for age; revealed 88% of patients were recorded as underweight, 92% as under height and 56% as under BMI.

Table1,Fig1:Showed the results of anthropometric measurements calculated by z-score; revealed a highly significant decrease in Wt, Ht, BMI, MAC and TSF in CKD patients when compared to the control

Table 2: Showed the results of laboratory data between patients and the controls; revealed a highly significant decrease in Hb, Hct, and serum Ca level. Significant decrease in total protein in CKD patients while no significant difference was detected regarding serum albumin. A highly significant increase in serum levels of urea creatinine, OX-HDL and hsCRP in patients group compared to the controls.

Table 3 ,Fig. 2: Showed that 100% of CKD patients on regular hemodialysis had high levels of both OX-HDL and hsCRP when compared to the controls.

Fig 3, Fig. 4: Represent the correlation between the OX-HDL serum levels and patients Wt and Ht, a highly significant positive correlation between OX-HDL and patients Wt and Ht were found.

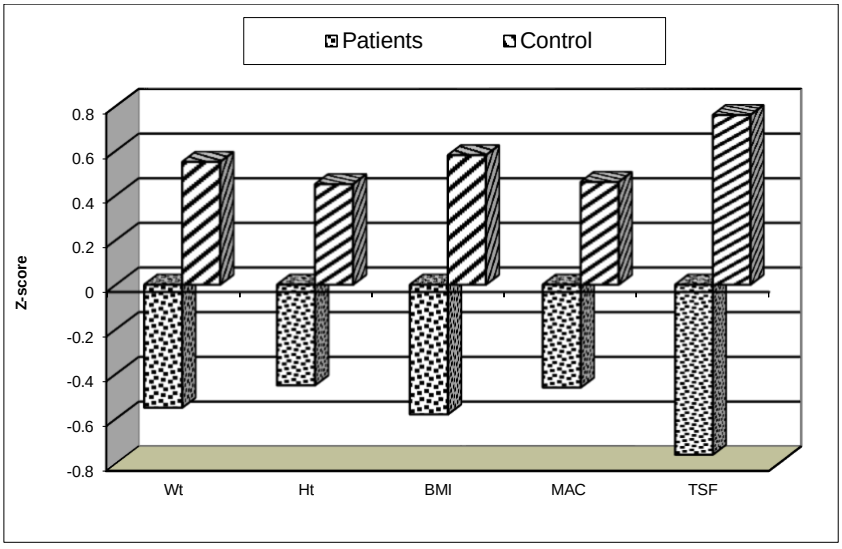
Table (1): Comparison between patients and control groups regarding weight, height and BMI centile for age.

Variable		Patients no=25		Control no=25		Chi-square		Sig
		no.	%	no.	%	X^2	P-value	
Weight centile	Under weight	22	88.00%	1	4.00%	35.507	0.01	HS
	Normal weight	3	12.00%	24	96.00%			
Height centile	Under height	23	92.00%	2	8.00%	35.28	0.01	HS
	Normal height	2	8.00%	23	92.00%			
BMI centile	Under BMI	14	56.00%	2	8.00%	13.235	0.01	HS

HS: highly significant

Table (2): Comparison between patients and the control group regarding anthropometric measurements calculated by Z-score**.**

Z-score for:	Patients no=25			Control no=25			"t" test		Sig.
	Mean	Min	Max	Mean	Min	Max	t	p-value	
Wt (kg)	-0.55	-0.45	-1.92	0.55	-1.45	1.60	-4.669	0.01	HS
Ht (cm)	-0.45	-0.55	-1.36	0.45	-1.04	2.25	-3.565	0.01	HS
BMI (kg/m^2)	-0.58	-0.58	-1.47	0.58	-0.87	2.40	-4.986	0.01	HS
MAC (cm)	-0.46	0.00	-0.58	0.46	-0.58	0.50	-3.657	0.01	HS
TSF(mm)	-0.76	-0.76	-1.29	0.76	-0.66	2.20	-8.302	0.01	HS



Fig(1): Comparison between patients and the control group regarding anthropometric measurements.

Table (3): Comparison between patients and control groups regarding laboratory data.

Variable	Patients no=25	Control no=25	" t" test		Sig
	Mean±SD	Mean±SD	t	p-value	
Hb (g/dl)	8.96±1.64	12.03±0.91	-8.177	0.01	HS
Hct (%)	27.72±3.55	36.43±2.51	-10.014	0.01	HS
Urea(mg/dl)	193.72±54.14	19.88±6.24	15.624	0.01	HS
Creatinine (mg/dl)	10.49±2.57	0.35±0.10	19.713	0.01	HS
Total protein (TP)	7.08±0.73	7.58±0.66	-2.539	0.05	S
Alb (g/dl)	4.17±0.78	4.25±0.40	-0.458	0.64	NS
OX-HDL (ug/l)	32.58±6.36	2.15±0.69	23.783	0.01	HS
hsCRP (ng/ml)	13900.00±315.99	278.84±89.59	49.087	0.01	HS

S:Significant

Table (4): Frequency and percentage of studied groups with high level of OX-HDL**.**

OX-HDL (ug/l)	Patients no=25		Control no=25		Chi-square test		Sig
	no.	%	no.	%	X^2	P-value	
Normal	0	0.00%	25	100.00%	50.000	0.01**	HS
High	25	100.00%	0	0.00%			
Total	25	100.00%	25	100.00%			

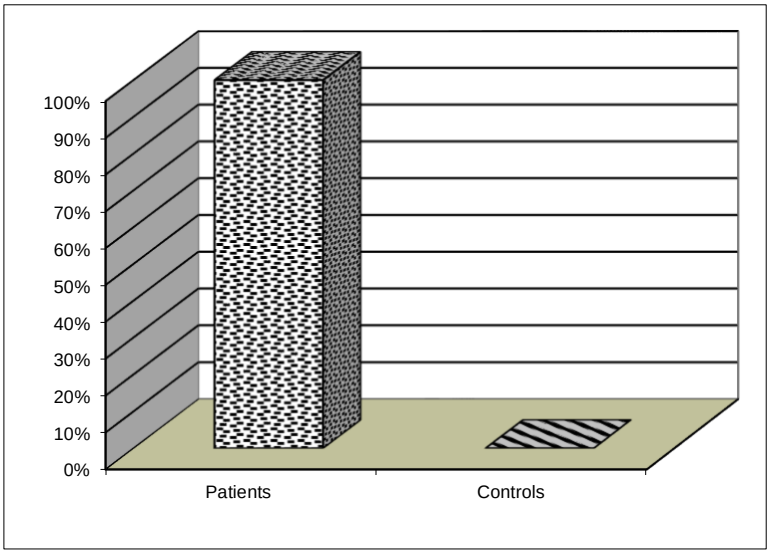


Figure (2): Comparison between patients and control groups regarding OX-HDL.

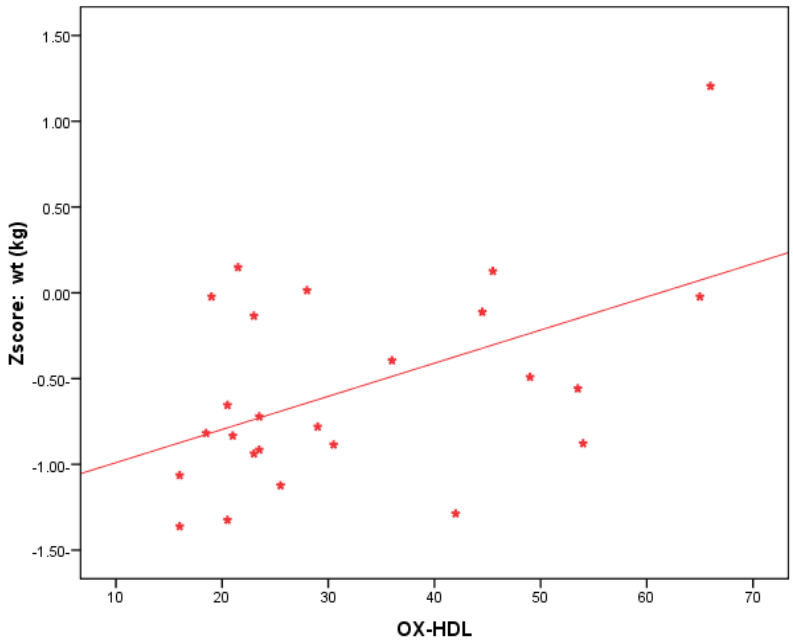


Figure (3): Correlation between OX-HDL and Z-score: Wt.

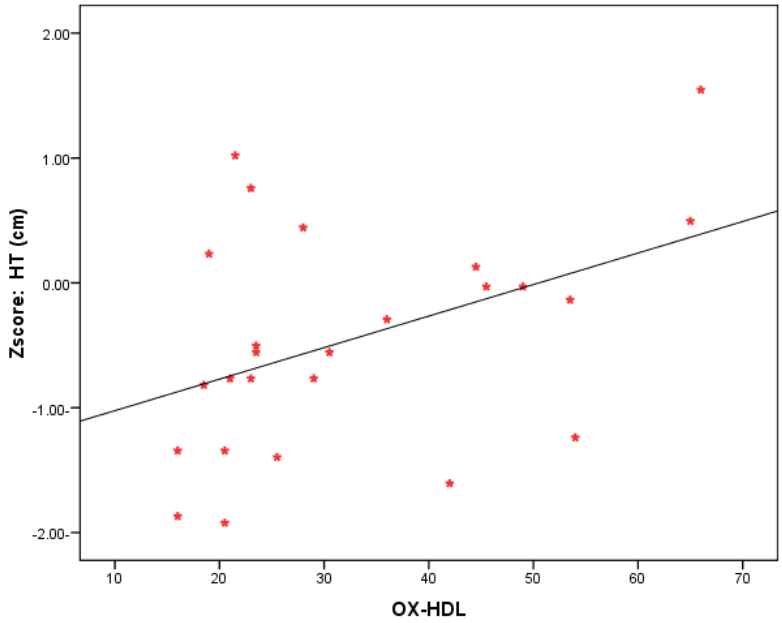


Figure (4): Correlation between OX-HDL and Z-score: Ht.

DISCUSSION

Human physical growth can be assessed by many methods including; anthropometry, bone age, dental age and radiological assessment. Anthropometry is the commonest method used. It is the simple technique of expressing quantitatively the form of the body[10].

Growth failure is the term that describes a growth rate below the appropriate growth velocity for age. A child is considered short if he or she has a height that is below the third percentile; alternatively, some define short stature as height less than 2 standard deviations below the mean [11]. In our study we assessed, centile for (Wt, Ht and BMI)and Z-score for (Wt, Ht, BMI, MAC and TSF). We found 88% of patients were recorded underweight, 92% under height and 56% under BMI. The present work demonstrates that anthropometric parameters including weight, height, BMI, MAC and TSF were affected in comparison to the control group. Also[12] found that 83.3% of the patient was short and the body weight in 46.7% of patients was under weight. Our results in agreement with those of another Egyptian study done by **Zahrane** etal.[13] on 64 children with CKD on conservative treatment with an age range of (0.5-21 years) they reported that the mean height was -3.7. **Sozeri *etal.*** [14]showed that growth deficit both in height and weight in dialysis children than the healthy control.

Growth failure in CKD children is multi-factorial. The age at onset of the disease, hormonal resistance, anemia, malnutrition, metabolic acidosis, renal osteodystrophy, and inadequate dialysis are all implicated [15]**.** We found that the mean Hb, Hct were significantly lower in CKD patients ,these results are in agreement with **Mahan and Warady** [16] stated that anemia is almost invariable feature of CKD and usually becomes manifest at a GFR less than 35 ml/min/1.73 m^2 .Anemia in patients with CKD is primarily the result of inadequate erythropoietin production by the failing kidneys. Other possible contributory factors include iron deficiency, folic acid or vitamin B12 deficiency, and decreased erythrocyte survival [17]**.** When we used serum albumin as a marker of malnutrition, the vast majority of patients presented values within the normal range, perhaps due to the fact that they were subjected to nutritional support as a part of the routine management in our unit. Our finding is in agreement with the studies done by **Morais** etal.and **Manandhar** etal. [18],[19] they revealed that the patients were having malnutrition and normal serum albumin.

On the other hand **Daniel** etal. **and Kaysen** etal. [20,21] found low serum albumin concentration in hemodialysis children.Hypoalbuminemia is the result of the combined effects of inflammation, inadequate protein and caloric intake in patients with chronic kidney disease.

hsCRP was assayed as a marker of chronic inflammation, we found that100% of CKD patients had high hsCRP, this in agreement with**Ortega**etal.,**Honda** etal. and **Abraham** etal.[22],[23],[24]they reported significant increase in hsCRP levels in maintenance hemodialysis patients. Elevated serum concentration of CRP is one of the most common nontraditional markers used to identify patients with chronic inflammation in CKD, and it has been used to detect cardiovascular risk, as it reflects the pro-inflammatory state. Most published studies showed consistent findings of a strong correlation between circulating CRP and pro-inflammatory cytokines, therefore, CRP reflects the hepatic response to high circulating pro-inflammatory cytokine levels in CKD patients [25]**.** Inflammation is an important cause of muscle wasting in CKD. Inflammation activates the ubiquitin–proteasome system, which leads to increased muscle breakdown. Inflammatory activity leads to catabolism of body cell mass. In acute disease, hypoalbuminemia can develop quite rapidly allowing body cell mass still to be relatively well preserved[26].

Unfortunately we found that 100% of our studied cases had high level of OX-HDL, this means that OX-HDL in the studied cases acts as a pro-inflammatory. HDL lose its protective capacity and even become pro-inflammatory in the setting of systemic inflammation. Because chronic inflammation and the state of oxidative stress are commonly sustained forms of protein damage in patients on maintenance hemodialysis, increased OX-HDL as dysfunctional HDL might enhance protein energy wasting in maintenance hemodialysis patients and might be involved in malnutrition-inflammation-atherosclerosis syndrome [27]. We found that significant positive correlations between OX-HDL levels with weight and height, while there was no significant correlation to BMI on the other hand **Murphy**et al[28]found that inverse correlations were found between OX-HDL and BMI, as OX-HDL may therefore not always be increased in patients with increased fat mass with concomitant oxidative stress but could appear in maintenance hemodialysis patients with low BMI and decreased fat. Several reports suggested that fat mass is associated with chronic inflammation and oxidative stress in ESRD patients, with a significantly higher truncal fat mass observed in ESRD patients with inflammation and protein energy wasting seeming to develop in overweight patients, particularly those with inflammation [27].**we concluded that,** malnutrition, and growth delay, are common finding in children with chronic kidney disease on regular hemodialysis, chronic inflammation may be missing link that actually ties protein energy malnutrition. HDL, in regular hemodialysis children loses its protective function as an anti-oxidant, anti-inflammatory and becomes pro-inflammatory as evidenced by high level of its oxidized form in all the studied patients.

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