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Research Article

ASSOCIATION BETWEEN SERUM NEOPTERIN LEVEL AND CAROTID ATHEROSCLEROSIS IN CHRONIC KIDNEY DISEASE PATIENTS

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ABSTRACT

Atherosclerotic cardiovascular disease is a major cause of morbidity and the leading cause of mortality in ESRD patients on maintenance HD.

Neopterin is expressed in endothelial cells, kidney epithelial cells, fibroblasts and vascular smooth muscle cells. Most studies dealing with effects of neopterin, provided evidence that interactions with reactive oxygen intermediate and the promotion of oxidative stress is a fundamental principle of neopterins mode of action and has been identified as a potential risk factor for CVD in dialysis patients.

The aim of the study is to evaluate the association between serum neopterin level and carotid atherosclerosis in chronic kidney disease patients.

The current study was conducted on 30 patients with ESRD on maintenance HD, 30 patients with CKD stage 5 not yet on dialysis and 20 healthy control subjects in Ain shams University Hospitals. All Subjects in this study were subjected to full history (stressing on etiology of renal failure, regimen of HD and duration on dialysis), Clinical examination and routine laboratory investigations as S. Creatinine, BUN, S.Calcium, S.phosphorus, S. PTH and lipid profile.

All subjects in the study were also subjected to the measurement of serum neopterin level, high sensitive CRP and IL 6.

All the study population were subjected to carotid duplex to measure carotid artery-intima media thickness (CA-IMT)also duplex ultrasound on brachial artery to measure flow mediated dilatation (FMD).

According to our results, CA-IMT measurement can be a more reliable indicator of atherosclerosis in CKD patients than FMD (%), as CA-IMT values were highest in group A (HD patients) and lowest in group C (healthy control subjects) with highly significant statistical difference between the three groups, whereas FMD (%) values showed non-significant statistical difference between the three groups.

Also CA-IMT values showed a statistically significant positive correlation with serum cholesterol levels in group B patients.

All inflammatory mediators showed a statistically significant difference between the three groups, also serum neopterin levels significantly increased with increasing IL 6 & HS CRP levels in group B patients.

Serum neopterin levels showed statistically significant positive correlation with serum BUN levels in both groups A & B patients, hence serum neopterin can be a more reliable indicator of inflammation in CKD and HD patients than IL 6 and HS CRP levels.

INTRODUCTION

Atherosclerotic cardiovascular disease is a major cause of morbidity and the leading cause of mortality in End Stage Renal Disease (ESRD) patients on maintenance hemodialysis (HD) *(Elena et al., 1994)*.

Endothelial dysfunction had constantly been observed in chronic renal failure (CRF) patients, and results in impaired

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modulation of vascular growth, dysregulation of vascular remodeling and altered anti-coagulant and anti-inflammatory properties of the endothelium *(Kosch et al., 2003)*.

Chronic inflammation is a common feature of ESRD *(Stenvinkel, 2001)*. It plays a key role in atherogenesis and atheroma development. Inflammatory mechanisms with atherosclerotic plaque formation can be triggered, maintained and enhanced by multiple factors such as oxidized low density lipoproteins (LDLs), increased reactive oxygen species and activated macrophages that induce synthesis of neopterin *(Roxbourgh et al., 1999)*.

Plasma neopterin originates as the oxidation product of 7,8-dihydroneopterin secreted by γ-interferon stimulated macrophages within atherosclerotic plaques, and is increasingly being used as a marker of inflammation during clinical management of patients with a range of disorders including atherosclerosis *(Gieseg et al., 2008)*.

Neopterin is expressed in endothelial cells, kidney epithelial cells, fibroblasts and vascular smooth muscle cells. Most studies dealing with effects of neopterin, provided evidence that interactions with reactive oxygen intermediate and the promotion of oxidative stress are fundamental principles of neopterins mode of action *(Hoffmann et al., 2003)*.

Neopterin levels have been shown to be elevated in patients with coronary artery disease (CAD) and associated with its severity, the complexity of atherosclerotic lesions and an increase in cardiovascular risk *(Sugioka et al., 2010)*.

Recent studies have shown a strong association of neopterin with cardiovascular disease (CVD). Sasaki *et al*., (2010) showed association of high levels with increased cardiac events rates. In a cross-sectional study, neopterin levels correlated with the extent of atherosclerosis, especially coronary and peripheral vascular disease (PVD) *(Hermus et al., 2011)*.

Neopterin has been identified as a potential risk factor for CVD in dialysis patients *(Avci et al., 2008)*.

The aim of the study is to evaluate the association between serum neopterin level and carotid atherosclerosis in chronic kidney disease patients.

SUBJECTS AND METHODS

This study is a cross sectional case control study and was conducted on 60 CKD patients and 20 healthy control subjects. The CKD patients were recruited from Nephrology Department of Ain Shams University hospital and divided into 2 major groups :

Group A: Included 30 clinically stable ESRD patients on maintenance HD.

Group B: Included 30 patients with CKD stage 5 (Not yet on dialysis).

The healthy control subjects were classified as **Group C.**
Informed consents were obtained from all participants.

Inclusion criteria

1. The patients were < 18 year old.
2. Group A patients were on regular HD for more than 6 months.
3. Group A patients were dialyzing 3 sessions/week with bicarbonate-containing dialysate using low flux hollow fiber dialyzers.

Exclusion criteria

1. Active Infections
2. DM
3. Obesity (BMI > 30)
4. Smoking
5. Malignancy

All Subjects in the study were subjected to:

1. Full history (stressing on etiology of renal failure, regimen of HD and duration on dialysis).
2. Clinical examination
3. Laboratory investigations:
   - S. Creatinine, blood urea nitrogen (BUN)
   - S.Calcium , S. phosphorus and S. PTH
   - Lipid profile
   - Serum Neopterin
   - Highly sensitive CRP
   - SerumIL 6
4. Radiologic assessment:
   - Carotid duplex to measure carotid artery intima-media thickness.
   - Duplex U/S on brachial artery to measure Flow mediated dilatation.

METHODS

Serum Neopterin level

Was based on the principle of enzyme-linked immunosorbent assay (ELISA). All Kits were stored at 2-8°C.

Neopterin ELISA kit is based on competitive binding of human Neopterin from serum samples and enzyme-labeled Neopterin to Neopterin specific antibodies immobilized on microtiter plates. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (blue color is inversely proportional to the amount of Neopterin present in the sample. The reaction is terminated by adding stopping solution which converts blue to yellow). Absorbance is then measured on an ELISA reader at 450nm and the concentration of Neopterin in samples and control is read off the standard curve.

Detection range of our kits was 50 pg/ml – 1000 pg/ml.

Highly sensitive CRP (Hs CRP):

Was based on the principle of a solid phase ELISA. Kits were stored at 2-8°C. All reagents were allowed to reach room temperature (18-22°C) before use. Patient serum and control serum were diluted 100 fold prior to use.

Obtained values of Hs CRP were in ng/ml. Patient samples with CRP concentrations greater than 10000 ng/ml were diluted 10 fold after the initial 100 fold dilution (total dilution 1:1000). Expected values of Hs CRP were from 68 to 8200 ng/ml.

Serum IL 6 level

IL6 is a 184 amino-acids polypeptide with potential O and N glycosylation sites. Kits were kept at 2-8 C. ELISA kit was designed to measure IL6. A monoclonal antibody specific for IL6 has been coated onto the wells of the microtiter strips provided. Samples including standards of known IL6 concentrations, control specimens were pipetted into these wells. During the first incubation, the standards or samples and a biotinylated monoclonal antibody specific for IL6 were simultaneously incubated. After washing, the enzyme Streptavidin - HRP, that binded the biotinylated antibody was added, incubated and washed. A TMB substrate solution was added which acted on the bound enzyme to induce a colored reaction product. The intensity of this colored product was directly proportional to the concentration of IL6 present in the samples.

***Carotid duplex to measure carotid artery intima-media thickness (CA-IMT) by specialized radiologist*:** It is a measure used to diagnose the extent of carotid atherosclerotic vascular disease. The test measures the thickness of the inner two layers of the carotid artery - the intima and media - and alerts physicians to any thickening when patients are still asymptomatic. Typically, normal CA-IMT at age 10 is approximately 0.4 to 0.5 mm, while from the first decade of life onward this progresses to 0.7 to 0.8 mm or more. Any values of more than 0.9 mm should be considered abnormal.

***Duplex Ultrasound on brachial artery to measure Flow mediated dilatation (FMD) by specialized radiologist*:** High-resolution ultrasonography was used to measure brachial artery diameter (BAD) at rest and following 5 mins of forearm occlusion (by sphygmomanometer cuff applied over the arm with pressure above 200 mm hg).

FMD(%) = [(BAD after deflation – Resting BAD) /Resting BAD] x 100 (%).

In general, the maximum change in brachial artery diameter is about 10%–20% in response to flow mediated vasodilatation. In patients with compromised endothelial function, the diameter change is likely to be less than 10%–20%.

Statistical analysis

- Clinical, laboratory and imaging characteristics of patients and healthy control subjects by Anova test.
- Classification of the 3 groups according to the gender by Chi-Square tests.
- Classification of the 3 groups according to the presence or absence of HTN by Chi-Square tests.
- Univariate analysis between serum neopterin level and clinical, inflammatory and imaging variables.
- Univariate analysis between serum IL 6 level and clinical, inflammatory and imaging variables.
- Univariate analysis between serum Hs CRP level and clinical, inflammatory and imaging variables.

Values for P less than 0.05 were considered significant and values less than 0.001 were considered highly significant.

RESULTS

The current study was conducted on 80 subjects, they were divided into 3 groups:

***Group A**:* consisted of 30 ESRD patients on maintenance HD (37.5 %).

***Group B**:* consisted of 30 CKD patients stage 5 not yet on dialysis (37.5 %).

***Group C**:* consisted of 20 healthy control subjects (25 %).

Table 1 Demographic data of the study groups

		Group A (HD patients) (n=30)	Group B (CKD stage 5 patients not on HD) (n=30)	Group C (Healthy Controls) (n=20)	P Value
Gender	Male: n(%)	24 (80%)	19 (63.3%)	17 (85%)	0.162
	Female: n(%)	6 (20%)	11 (36.7%)	3 (15%)	
Age (years)	Mean ± SD	49.4±11.1	53.9±14	31.3±7.5	0.000*
	Range	22 - 64	18 - 83	26 – 50	
HTN	Yes : N (%)	20 (66.7%)	16 (53.3%)	0 (0.0%)	0.000
	No : N (%)	10 (33.3%)	14 (46.7%)	20 (100%)	

Group A (Hemodialysis group) included 30 patients; 24 males (80%) and 6 females (20 %). Their age range was from 22-64 years with mean age 49.4±11.1 years. Twenty patients were hypertensive (66.7 %) while the remaining 10 patients were normotensive (33.3%).

Group B (CKD stage 5 not yet on HD) included 30 patients; 19 males (63.3%) and 11 females (36.7%). Their age range was from 18-83 years with mean age 53.9±14 years. Sixteen patients were hypertensive (53.3%) while the remaining 14 patients were normotensive (46.7 %).

Group C (Healthy controls) included 20 subjects; 17 males (85%) and 3 females (15 %). Their age range was from 26-50 years with mean age 31.3±7.5 years. All of them were normotensive (100%).

* By performing Post Hoc Test, there was no statistical difference between group A (HD patients) and group B (CKD patients not on dialysis). There was a statistical difference only between healthy control subjects and the patients.

Table 2 Etiology of Renal failure in ESRD patients (Group A patients):

Etiology of ESRD	HTN	17 (56.7%)
	ADPKD	1 (3.3%)
	Systemic Lupus Erythematosis	1 (3.3%)
	Obstructive uropathy	5 (16.7%)
	Chronic Glomerulonephritis	2 (6.7%)
	Reflux nephropathy	2 (6.7%)
	Recurrent UTI	1 (3.3%)
	Unknown	1 (3.3%)

Group A patients are divided according to etiology of ESRD into 17 hypertensive patients (56.7%), one patient with Autosomal dominant polycystic kidney disease (ADPKD) (3.3 %), one with systemic lupus erythematosis (3.3 %), five patients with obstructive uropathy (16.7%), two patients with chronic glomerulonephritis (6.7%), two patients with reflux nephropathy (6.7%), one patient with recurrent urinary tract infection (UTI) (3.3 %) and one with unknown etiology (3.3%).

As regards to KFTs : Both BUN and Creatinine showed highly significant statistical difference between the 3 groups **(P value 0.000)** and when we performed post hoc test, there was a statistical difference between group A and B and also between each of the two groups and the control group.

Table 3 Laboratory characteristics of subjects in the different groups

		Group A (HD patients) (n=30)	Group B (CKD stage 5 patients not on HD) (n=30)	Group C (Healthy Controls) (n=20)	P value
BUN (mg/dl)	**Mean±SD**	79.17±15.69	58.97±11.58	12.35±2.37	0.000
	Range	52 – 118	38 - 84	8 – 16	
Creat. (mg/dl)	**Mean±SD**	10.20±1.75	5.52±1.01	0.76±0.14	0.000
	Range	7.6 – 13.6	3.6 - 8	0.5 – 1	
Calcium (mg/dl)	**Mean±SD**	8.42±0.71	8.73±0.67	9.17±0.39	0.000
	Range	7.1 – 10.7	7.8 – 10.2	8.2 – 9.7	
Po4 (mg/dl)	**Mean±SD**	5.42±1.15	5.54±1.03	3.64±0.45	0.000
	Range	2.5 – 7.4	2.8 – 7.4	2.7 – 4.4	
PTH (pg/ml)	**Mean±SD**	535.9±444.4	255.6±125.6	40.8±15.62	0.000
	Range	21.7 – 1937	35.5 - 490	18 – 65	

As regards to bone parameters: All the 3 parameters showed highly significant statistical difference between the 3 groups **(P value 0.000)**, however serum PTH level was the only parameter that showed statistical difference between group A and B when performing post hoc test.

By performing post hoc test, both serum neopterin levels and IL 6 levels showed no statistical difference between group A and group B patients.

Table 4 Lipid profile of subjects in the different groups

		Group A (HD patients) (n=30)	Group B (CKD stage 5 patients not on HD) (n=30)	Group C (Healthy Controls) (n=20)	P value
Chol. (mg/dl)	**Mean±SD**	193.77±21.48	152.17±34.79	170±19.08	0.000
	Range	155 – 240	78 - 267	130 – 203	
TG (mg/dl)	**Mean±SD**	123.07±14.94	147.63±53.11	86±18.9	0.000
	Range	89 – 160	82 - 272	40 – 122	
HDL (mg/dl)	**Mean±SD**	36.33±5.98	52.87±17.32	46.35±10.95	0.000
	Range	27 – 50	13 - 80	16 – 67	
LDL (mg/dl)	**Mean±SD**	140.37±24.69	136.37±33.18	108.5±28.47	0.001
	Range	86 – 196	73 - 176	62 – 164	

As regards to lipid profile: Cholesterol ,TG, HDL and LDL all showed highly significant statistical difference between the 3 groups with **(P values 0.000, 0.000, 0.000, 0.001 respectively)**.

By performing post hoc test, all parameters of lipid profile showed statistical difference in between the 3 groups except for serum HDL levels between groups B & C and serum LDL levels between groups A & B.

HsCRP was 7495±2143.9, 5778.3±2286 and 4465±1551.4 ng/ml in groups A,B and C respectively with highly significant statistical difference between the 3 groups **(P value 0.000)**.

CA-IMT was 1.24±0.17, 1.1±0.2 and 0.69±0.24 mm in groups A,B and C respectively with highly significant statistical difference between the 3 groups **(P value 0.000)**.

Table 5 Inflammatory mediators of subjects in the different groups

		Group A (HD patients) (n=30)	Group B (CKD stage 5 patients not on HD) (n=30)	Group C (Healthy Controls) (n=20)	P value
Neopterin (pg/ml)	**Mean±SD**	509.3±254.5	445±188.1	134.3±35.4	0.000
	Range	110 – 1000	110 - 1000	80 – 200	
IL6 (pg/ml)	**Mean±SD**	166±69.2	188.5±97.6	39.3±32.7	0.000
	Range	85 – 370	65 - 400	10 – 100	
HsCRP (ng/ml)	**Mean±SD**	7495±2144	5778±2286	4465±1551	0.000
	Range	3000-10000	2500-10000	1500-7000	

Serum neopterin level was 509.3±254.5 pg/ml with minimum value 110 pg/ml and maximum 1000 pg/ml in group A patients, 445±188 pg/ml with also minimum value of 110 pg/ml and maximum one of 1000 pg/ml in group B patients. Neopterin level was 134.2±35.4 pg/ml with minimum value of 80 pg/ml and maximum 200 pg/ml in group C (control subjects). Neopterin levels showed highly significant statistical difference between the 3 groups with **(P value of 0.000)**.

IL 6 was 166±69.2, 188.5±97.6 and 39.3±32.7 pg/ml in groups A,B and C respectively with highly significant statistical difference between the 3 groups **(P value 0.000)**.

Resting brachial artery diameter was 4.75±0.88, 5.05±0.81and 4.76±0.5 mm in groups A,B and C respectively. It showed no significant statistical difference between the 3 groups **(P value 0.266)**.

Brachial artery diameter after deflation was 5.26±1.06, 5.65±0.73 and 5.29±0.6 mm in groups A,B and C respectively. It showed no significant statistical difference between the 3 groups **(P value 0.162)**. Flow mediated dilatation (%) was 10.7±7.3, 12.8±10 and 11.2±7.1 in groups A,B and C respectively. It showed no significant statistical difference between the 3 groups **(P value 0.614)**.

Table 6 Imaging characteristics of subjects in the different groups

		Group A (HD patients) (n=30)	Group B (CKD stage 5 patients not on HD) (n=30)	Group C (Healthy Controls) (n=20)	P value
CA-IMT (mm)	Mean±SD	1.24±0.17	1.1±0.2	0.69±0.24	0.000
	Range	0.9 – 1.6	0.6 – 1.5	0.4 – 1.2	
BAD [resting] (mm)	Mean±SD	4.75±0.88	5.05±0.81	4.76±0.5	0.266
	Range	3.4 – 7.5	3.6 – 6.3	3.7 – 5.5	
BAD [after deflation] (mm)	Mean±SD	5.26±1.06	5.65±0.73	5.29±0.6	0.162
	Range	3.6 – 9	4.1 – 7.1	3.9 – 6	
FMD (%)	Mean±SD	10.69±7.31	12.77±10.03	11.19±7.12	0.614
	Range	2.3 – 34.1	3.28 – 38.9	4.1 – 30.4	

Table 7 Correlations between serum neopterin level and clinical and inflammatory variables in the different groups

	Serum Neopterin level					
	Group A (HD patients) (n=30)		Group B (CKD stage 5 patients not on HD) (n=30)		Group C (Healthy Controls) (n=20)	
	r value	P-value	r value	P-value	r value	P-value
Age	-0.199	0.291	-0.063	0.740	0.296	0.205
BUN	0.433	**0.017**	0.388	**0.034**	-0.069	0.773
Creatinine	0.432	**0.017**	0.340	0.066	-0.513	**0.021**
S.Calcium	0.288	0.123	0.023	0.903	-0.251	0.287
S.phosphorus	0.028	0.884	-0.079	0.679	0.340	0.142
PTH	-0.063	0.740	0.315	0.090	0.272	0.247
S.Cholesterol	-0.242	0.198	-0.015	0.938	0.231	0.327
S.Triglycerides	0.065	0.731	0.219	0.245	-0.321	0.168
HDL	0.353	0.056	-0.263	0.160	0.002	0.993
LDL	-0.094	0.623	0.060	0.753	0.427	0.061
CA IMT	0.048	0.801	-0.080	0.674	0.457	**0.043**
FMD (%)	0.191	0.313	0.176	0.353	-0.401	0.080
Dialysis duration	0.240	0.201				
IL 6	-0.023	0.904	0.480	**0.007**	0.298	0.203
Hs CRP	0.145	0.444	0.580	**0.001**	0.338	0.145

Serum neopterin levels showed statistically significant positive correlation of weak strength with both BUN and Creatinine levels in group A patients **(P value in both 0.017)** (Table 7, Figure 1).

There was a trend of positive correlation between serum neopterin levels and dialysis duration, CA-IMT, FMD but with non-statistical significance (Table 7, Figures 2&3).

Serum neopterin level showed negative correlation with serum IL 6 level in group A patients but of non-statistical significance **(P value 0.904).**

Serum neopterin level showed positive correlation with Hs CRP level in group A patients but of non-statistical significance **(P value 0.444).**

Serum neopterin level showed statistically significant positive correlation of weak strength with BUN levels in group B patients **(P value 0.034),** while serum neopterin levels showed positive correlation with serum creatinine levels in this group but of non-statistical significance with (**P value 0.066).**

Serum neopterin level showed negative correlation with CA IMT in mm in group B patients but of non-statistical significance with (**P value 0.674).**

Serum neopterin level showed positive correlation with FMD (%) in group B patients but of non-statistical significance with (**P value 0.353).**

Serum neopterin levels showed statistically significant positive correlation with both serum IL 6 levels and Hs CRP levels in group B patients with **P values 0.007 and 0.001** respectively.

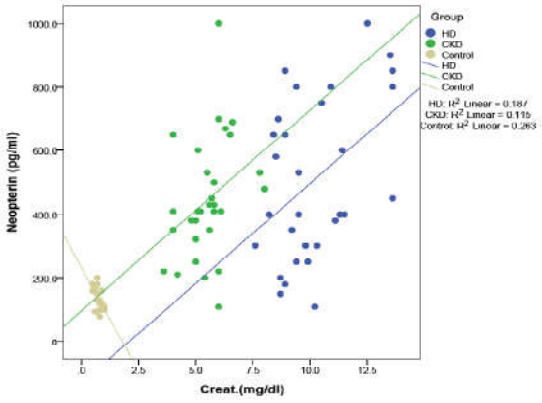

Figure 1 Correlation between serum neopterin level and serum creatinine in the different groups

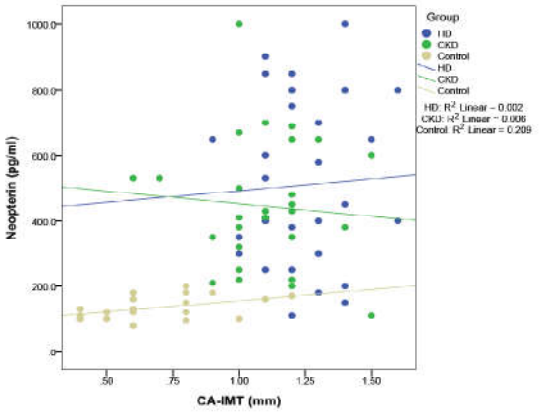

Figure 2 Correlation between serum neopterin level and CA IMT in the different groups

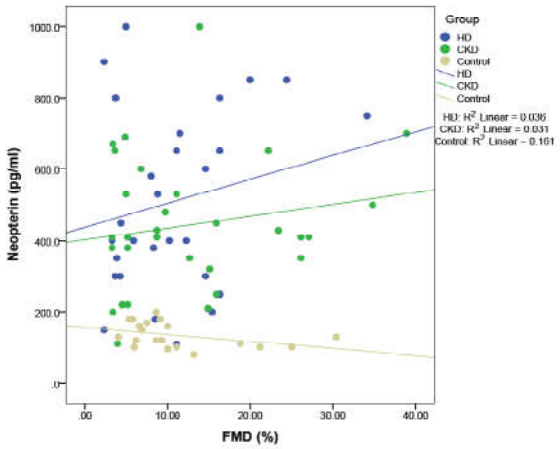

Figure 3 Correlation between serum neopterin level and FMD(%) in the different groups

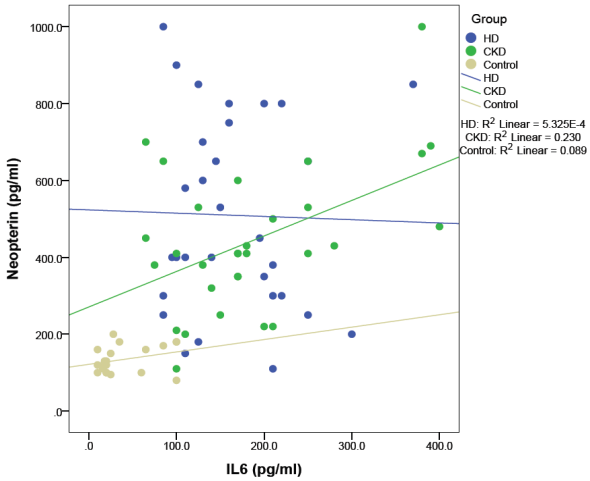

Figure 4 Correlation between serum neopterin level and IL 6 level in the different groups

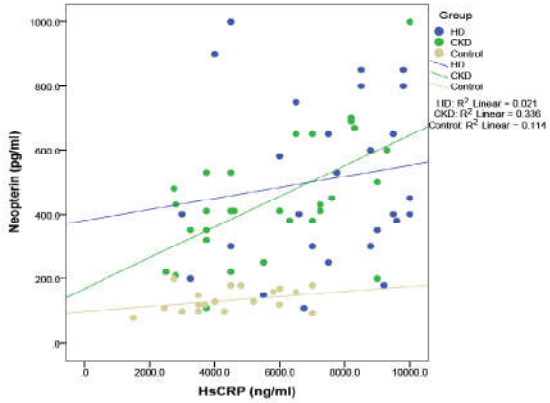

Figure 5 Correlation between serum neopterin level and Hs CRP level in the different groups

SerumIL 6 levels showed a trend of negative correlation with CA-IMT and serum HDL levels and a positive correlation with HS CRP levels in groups A and B patients. All these correlations were not significant except the correlation between serum IL 6 level and serum HDL levels in group A patients **(P value 0.034)** (Table 8, Figures 7&9).

Table 8 Correlations between serum IL 6 level and clinical and inflammatory variables in the different groups

	SerumIL 6 level					
	Group A (HD patients) (n=30)		Group B (CKD stage 5 patients not on HD) (n=30)		Group C (Healthy Controls) (n=20)	
	r value	P-value	r value	P-value	r value	P-value
Age	0.062	0.746	-0.184	0.330	0.620	**0.004**
BUN	0.146	0.441	0.076	0.689	0.071	0.767
Creatinine	0.081	0.671	0.305	0.102	-0.549	**0.012**
S.Calcium	-0.144	0.447	0.089	0.641	-0.500	**0.025**
S.phosphorus	-0.029	0.880	-0.105	0.580	0.265	0.258
PTH	-0.047	0.807	0.133	0.483	0.026	0.912
S.Cholesterol	0.263	0.160	-0.139	0.463	0.028	0.907
S.Triglycerides	0.054	0.778	0.291	0.119	0.281	0.231
HDL	-0.389	**0.034**	-0.031	0.870	-0.429	0.059
LDL	0.323	0.082	0.244	0.193	0.284	0.225
CA IMT	-0.014	0.942	-0.117	0.539	0.594	**0.006**
FMD (%)	0.266	0.155	-0.071	0.709	-0.260	0.268
Dialysis duration	-0.091	0.633				
S.Neopterin	-0.023	0.904	0.480	**0.007**	0.298	0.203
Hs CRP	0.024	0.901	0.176	0.352	0.073	0.758

Serum IL 6 levels showed a positive correlation with FMD (%) in group A patients and a negative correlation with FMD (%) in both groups B and C but all are of non- statistical significance (Table 8, Figure 8).

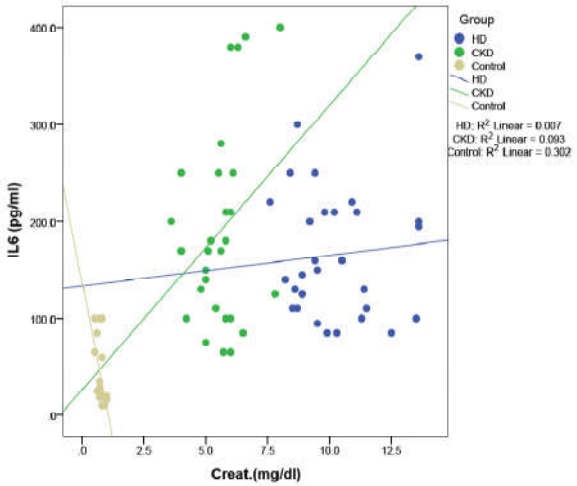

Figure 6 Correlation between serum IL 6 level and serum creatinine in the different groups

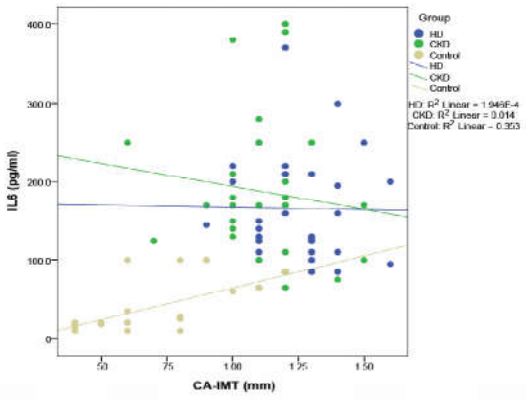

Figure 7 Correlation between serum IL 6 level and CA IMT in the different groups

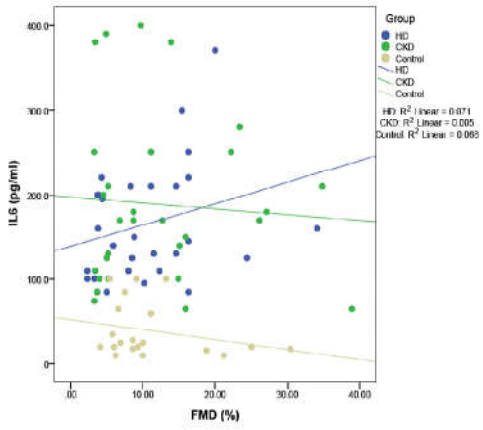

Figure 8 Correlation between serum IL 6 level and FMD (%) in the different groups

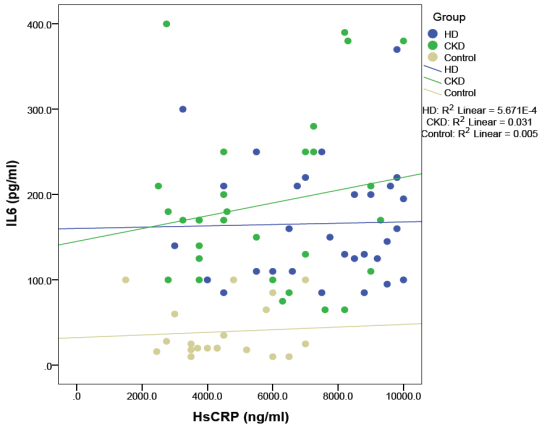

Figure 9 Correlation between serum IL 6 level and Hs CRP level in the different groups

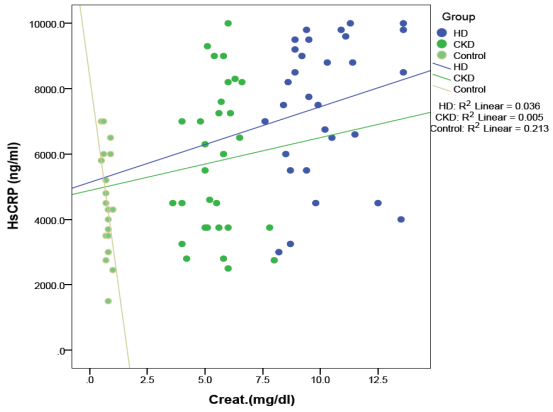

Figure 10 Correlation between Hs CRP level and serum creatinine in the different groups

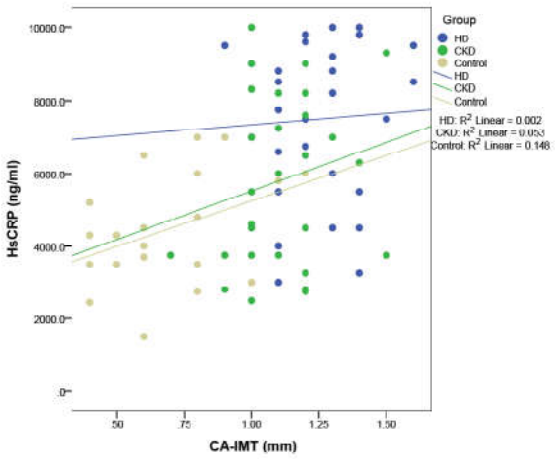

Figure 11 Correlation between Hs CRP level and CA IMT in the different groups

Table 9 Correlations between serum Hs CRP level and clinical and inflammatory variables in the different groups

	Serum Hs CRP level					
	Group A (HD patients) (n=30)		Group B (CKD stage 5 patients not on HD) (n=30)		Group C (Healthy Controls) (n=20)	
	r value	P-value	r value	P-value	r value	P-value
Age	-0.136	0.474	0.328	0.077	0.434	0.056
BUN	-0.047	0.804	0.458	**0.011**	-0.405	0.076
Creatinine	0.189	0.317	0.072	0.707	-0.461	**0.041**
S.Calcium	-0.117	0.539	0.183	0.332	-0.487	**0.029**
S.phosphorus	0.018	0.924	-0.180	0.342	0.253	0.282
PTH	-0.197	0.297	0.372	**0.043**	0.488	**0.029**
S.Cholesterol	0.004	0.984	0.236	0.209	-0.180	0.448
S.Triglycerides	-0.112	0.556	0.298	0.109	-0.150	0.528
HDL	-0.330	0.075	-0.422	0.020	-0.407	0.075
LDL	0.075	0.693	0.106	0.577	0.102	0.668
CA IMT	0.049	0.798	0.229	0.223	0.385	0.094
FMD (%)	0.005	0.979	0.085	0.656	-0.204	0.389
Dialysis duration	-0.008	0.965				
S.Neopterin	0.145	0.444	0.580	**0.001**	0.338	0.145
IL 6	0.024	0.901	0.176	0.352	0.073	0.758

Serum Hs CRP levels showed statistically significant positive correlation of weak strength with both BUN levels and PTH levels in group B patients with **P values 0.011 and 0.043** respectively.

Serum Hs CRP levels showed a trend of positive correlation with serum creatinine levels, CA-IMT and FMD (%) in both groups A & B patients but of non-statistical significance (Table 9, Figures 10-12).

CA-IMT showed statistically significant positive correlation of weak strength with serum HDL levels in group A patients **(P value 0.006).**

CA-IMT showed statistically significant negative correlation of weak strength with serum LDL levels in group A patients **(P value 0.006).**

CA-IMT showed statistically significant positive correlation of moderate strength with age in group B patients **(P value 0.000)**

while it showed positive correlation in group A patients but of non- statistical significance.

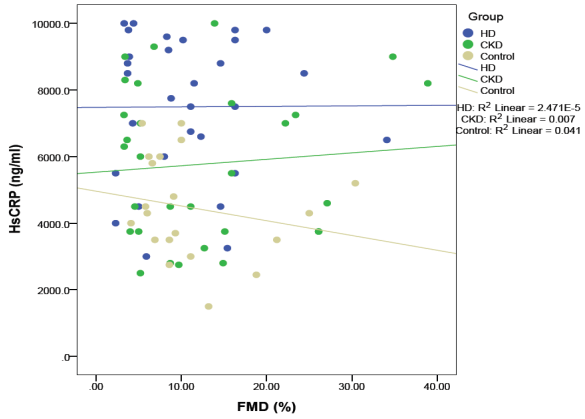

Figure 12 Correlation between Hs CRP level and FMD (%) in the different groups

Flow mediated dilatation (%) showed negative correlation with all of BUN levels, serum creatinine levels, CA IMT and duration of dialysis in years in group A patients but of non-statistical significance.

Also flow mediated dilatation (%) showed negative correlation with all of the age of the patients, serum creatinine level, cholesterol & triglycerides levels, serum LDL levels and CA IMT in group B patients but of non-statistical significance.

DISCUSSION

Recent data suggested that atherosclerosis is a chronic inflammatory disorder. Inflammatory cells including macrophages and activated T lymphocytes infiltrate atherosclerotic lesions *(Tousoulis et al., 2003)*.

Measuring serum neopterin levels is a useful tool for the biochemical monitoring of the activation of cell-mediated (Th1-type) immune response.

Table 10 Correlations between CA IMT and clinical and inflammatory variables in the different groups

	CA IMT (in mm)					
	Group A (HD patients) (n=30)		Group B (CKD stage 5 patients not on HD) (n=30)		Group C (Healthy Controls) (n=20)	
	r value	P-value	r value	P-value	r value	P-value
Age	0.296	0.112	0.692	**0.000**	0.836	**0.000**
BUN	0.150	0.430	0.114	0.548	-0.357	0.122
Creatinine	0.177	0.349	-0.137	0.470	-0.632	**0.003**
S.Calcium	0.099	0.602	0.001	0.996	-0.586	**0.007**
S.phosphorus	0.136	0.474	0.214	0.256	0.597	**0.005**
PTH	-0.038	0.840	0.021	0.913	-0.009	0.970
S.Cholesterol	-0.138	0.469	0.463	**0.010**	0.038	0.873
S.Triglycerides	0.193	0.308	0.125	0.510	0.074	0.758
HDL	0.489	**0.006**	-0.088	0.645	-0.509	**0.022**
LDL	-0.406	**0.026**	0.145	0.444	0.355	0.124
FMD (%)	-0.244	0.195	-0.199	0.293	-0.520	**0.019**
Dialysis duration	-0.144	0.447				
S.Neopterin	0.048	0.801	-0.080	0.674	0.457	**0.043**
IL 6	-0.014	0.942	-0.117	0.539	0.594	**0.006**
Hs CRP	0.049	0.798	0.229	0.223	0.385	0.094

CA-IMT showed statistically significant positive correlation of weak strength with serum cholesterol levels in group B patients **(P value 0.010).**

Neopterin is not only a marker for an activated Th1-mediated cellular immune system, but it also augments the production, release, or effect of reactive oxygen species, and can enhance LDL oxidation.

Table 11 Correlations between FMD (%) and clinical and inflammatory variables in the different groups

	FMD (%)					
	Group A (HD patients) (n=30)		Group B (CKD stage 5 patients not on HD) (n=30)		Group C (Healthy Controls) (n=20)	
	r value	P-value	r value	P-value	r value	P-value
Age	0.157	0.408	-0.271	0.147	-0.233	0.322
BUN	-0.261	0.163	0.177	0.350	0.236	0.316
Creatinine	-0.079	0.679	-0.132	0.486	0.385	0.093
S.Calcium	-0.157	0.406	0.181	0.338	0.319	0.170
S.phosphorus	0.158	0.404	-0.129	0.497	-0.415	0.069
PTH	0.198	0.295	0.086	0.652	0.194	0.413
S.Cholesterol	0.021	0.912	-0.300	0.108	-0.173	0.465
S.Triglycerides	-0.261	0.163	-0.169	0.373	0.146	0.538
HDL	-0.126	0.506	0.193	0.306	0.151	0.526
LDL	0.079	0.677	-0.107	0.575	-0.002	0.993
CA IMT	-0.244	0.195	-0.199	0.293	-0.520	**0.019**
Dialysis duration	-0.089	0.638				
S.Neopterin	0.191	0.313	0.176	0.353	-0.401	0.080
IL 6	0.266	0.155	-0.071	0.709	-0.260	0.268
Hs CRP	0.005	0.979	0.085	0.656	-0.204	0.389

Elevated serum neopterin levels were previously demonstrated in patients with atherosclerosis *(Gurfinkel et al., 1999)*.

The present study was conducted upon 80 patients (60 males, 20 females; mean age 46.6 ± 14.6 years) in order to study the association between serum neopterin level and carotid atherosclerosis in CKD patients. We also studied the relationship between Hs-CRP and IL6, as markers of inflammation, and CA-IMT.

In our study, patients were divided into 3 groups: **Group A**: consisted of 30 ESRD patients on maintenance hemodialysis, **Group B**: consisted of 30 CKD patients stage 5 not on dialysis and **Group C**: consisted of 20 healthy control subjects.

According to presence or absence of HTN, our study population consisted of 20 hypertensive patients (66.7 %) and 10 normotensive patients (33.3%), 16 hypertensive patients (53.3%) and 14 normotensive patients (46.7 %), 20 normotensive subjects in groups A, B and C respectively.

The mean age of the subjects in our study was 49.4±11.1 years, 53.9±14 years and 31.3±7.5 years in groups A,B and C respectively. The Age of the patients showed positive correlation with CA IMT reflecting the aging effect on atherosclerosis progression.This agrees with Zaki *et al*., (2008), Szeto *et al*., (2007) and Erten *et al*., (2005) as in these studies, the age of the subjects showed positive correlation with CA IMT.

Serum neopterin levels significantly correlated with the extent of atherosclerosis in patients with acute coronary events. Furthermore, some studies demonstrated a correlation between serum neopterin concentrations and the extent of carotid atherosclerosis as assessed by using a plaque-scoring system *(Gurfinkel et al., 1999)*.

Accordingly, a circulating neopterin level could be a valuable marker of atherosclerosis. Previous studies reported that IMT values of the carotid arteries in hemodialysis patients were significantly higher than in healthy controls. Moreover, some studies demonstrated that there was a positive correlation between carotid IMT and serum neopterin levels in patients on chronic hemodialysis. Therefore, neopterin levels could be associated with the correlation between serum neopterin level and HD duration.

Serum neopterin level showed positive correlation with HS CRP level. This agrees with Formanowicz (2012) study in which there was also positive correlation between both, suggesting an overall state of active inflammation in our patients.

We found also positive correlation of highly statistical significance between serum neopterin level and HS CRP level in group B patients (CKD patients not on dialysis). This agrees with both Yilmaz *et al*., (2011) and Zaki *et al*., (2008) as in both studies, there was also positive correlation of statistical significance between serum neopterin level and HS CRP level in CKD patients not on dialysis.

Hs CRP mean values in our study were higher in HD patients than in CKD patients not yet on dialysis than in healthy control subjects. Similar to our results , various studies as Tbahriti *et al*., (2013), Yilmaz *et al*., (2011) and Zaki *et al*., (2008) found that CRP levels were higher in HD patients than in CKD patients with highly significant statistical difference between all the groups of the studies.

Hyperlipidemia was found to be associated with increasing the stages of CKD. The mean levels of total cholesterol, triglycerides and LDL were significantly increased in CKD and HD groups than in control group. This is similar to Zaki, **(**2008**)** study which showed also that levels of total cholesterol, triglycerides and LDL were significantly increased in CKD and HD groups than in healthy control subjects.

Also mean HDL values (the good cholesterol) in our study were significantly lower in CKD patients and HD groups than in control group. This is similar also to Zaki, *et al*., (2008) study.

These findings in addition to increased inflammation demonstrated before, also explain increased cardiovascular morbidity in ESRD patients with the help of some other mechanisms such as endothelial dysfunction.

Carotid atherosclerosis in our study increased in both HD patients and CKD patients (not yet on dialysis) than in the control group with CA-IMT values 1.24±0.17, 1.1±0.2 and 0.69±0.24 mm in groups A,B and C respectively.

When we studied the association between serum neopterin level and carotid atherosclerosis, CA IMT showed positive correlation with serum neopterin level in group A patients (HD patients) but of non-statistical significance. However, in the study done by Formanowicz (2012), CA IMT showed positive correlation with serum neopterin level but of statistical significance, this may be due to smaller sample size in our study. This finding indicates that neopterin can be used as a marker for the activity of atherosclerosis in hemodialysis patients.

Although we found positive correlation between HS CRP and CA IMT, this showed no statistical significance. Lai *et al*., (2014), Sathi *et al*, (2014), and Szeto *et al*, (2007), found positive significant statistical correlation between CA IMT and Hs CRP level, this is because Lai *et al*., (2014), studied five stages of CKD, and Sathi *et al*., (2014), and Szeto *et al*., (2007), performed their studies on larger sample size.

The mean values of FMD (%) were higher in CKD patients not yet on dialysis and healthy control subjects than in HD patients. FMD (%) values were 10.7±7.3, 12.8±10 and 11.2±7.1 in groups A,B and C respectively but with no statistical significance. In Yilmaz *et al*., (2011) study, the mean values of FMD (%) were lower in CKD stage 5 patients than in other CKD stages but with highly statistical significance. This may be attributed to smaller sample size in our study.

So, it is recommended to have the following

1. Larger samples may be required to study the association between flow mediated dilatation of brachial artery and inflammatory variables in CKD and ESRD patients.
2. It is advised to take samples of patients in the different 5 stages of CKD for better evaluation of the relation between increased stage of CKD and increasing severity of atherosclerosis.

Also taking samples of patients from the different 5 stages of CKD will allow us to study the accurate relation between

decreased GFR in the patients and higher inflammatory variables.

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