Circulating Endothelial Cells and Cardiovascular Risk in Chronic Kidney Disease and Hemodialysis Patients

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Abstract
Chronic kidney disease is a potent risk factor for cardiovascular disease and it remains the leading cause of morbidity and mortality in patients with chronic kidney disease. As renal function declines, there are a host of abnormalities that develop, including changes in coagulation, fibrinolysis, lipids, endothelial dysfunction, and many other factors that have been related to cardiovascular disease. This work was conducted with the aim to study circulating endothelial cells count in chronic kidney disease and hemodilaysis patients and correlate this to other markers of cardiovascular risk in this population. The study included 50 subjects classified as follows: Group I included 20 patients with chronic kidney disease not yet on dialysis, group II included 20 patients on maintenance hemodialysis and 10 age and sex matched individual used as a control (group III). All included individual were subjected to complete blood count, renal function tests, estimation of glomerular filtration rate by Modification of Diet in Renal Disease formula for non –dialysis patients, complete lipid profile, high sensitive C-reactive protein, and erythrocyte sedimentation rate, and detection of circulating endothelial cells count using flow cytometry. The study showed increased count of circulating endothelial cells and high sensitive C-reactive protein in both patients groups than the control and it was higher in patients on dialysis than those on conservative treatment. There was a statistically positive correlation between erythrocyte sedimentation rate, high sensitive C-reactive protein, Triglycerides and circulating endothelial cells in the three groups. So, detection of circulating endothelial cells count might offer a mean for recognizing chronic kidney disease patients at risk of cardiovascular events.
Keywords: *chronic kidney disease, hemodialysis, cardiovascular risk, circulating endothelial cells.*

Introduction

Chronic kidney disease is a worldwide public health problem which is capable of producing a wide variety of complications most important of them are cardiovascular complications.[1]

Chronic kidney disease is a potent risk factor for CVD and cardiovascular disease remains the leading cause of morbidity and mortality in patients with CKD and may account for 50% of all deaths. Individuals requiring dialysis treatment have cardiovascular mortality rates that are 10 to 20 times greater than age- and sex-matched controls in the general population.[2]

As renal function declines, there are a host of abnormalities that develop, including changes in coagulation, fibrinolysis, lipids, endothelial dysfunction, and many other factors that have been related to CVD.[3]

Among several novel risk factors, persistent inflammation (usually recognized by elevated serum levels of C-reactive protein (CRP)), has attracted much interest. Although the association between CRP and vascular disease has been recognized for several decades, the concept of micro inflammation is at present a hot topic in renal literature.[4]

Elevated CRP is wildly considered as a marker of the underlying inflammatory process in end stage renal disease (ESRD).[5]

The endothelial cell is critically involved in neovascularization and in the regulation of the structure and function of established blood vessels. Endothelial cells generate signaling molecules such as nitric oxide (NO), prostacyclin and endothelin which serve diverse autocrine and paracrine functions, and they form a monolayer which modulates local hemostasis and thrombolysis and provides a nonpermeable barrier protecting the underlying vascular smooth muscle from circulating growth-promoting factors. The vascular endothelium regulates vessel tone by releasing vasoactive substances such as nitric oxide (NO).[6,7]

Endothelial dysfunction is commonly observed along the CKD spectrum, being an obligatory prodromal phase in the atherosclerosis process that likely precedes other cardiovascular complications.[8]

The circulating endothelial cells include endothelial progenitor cell (EPC) which are bone marrow-derived, and inflammatory endothelial cells, which are thought to be detached from the vessel walls and thrown to the circulation as a result of vascular injury. Normally, in response to ischemic insult and cytokine stimulation, EPC are mobilized from the bone marrow to act as repair cell in response to endothelial injury indeed, low levels of EPC predicted the occurrence of cardiovascular events and death in coronary artery disease patients.[9]

If the end result of a variety of insults, hypertension, hyperlipidemia, oxidative stress, was endothelial damage, then the number of detached endothelial cells circulating in blood may be a sensitive and specific measure of endothelial injury. The number of these detached endothelial cells may not only be a direct measure of the severity of the insult, but also a direct measure of the ability of an individual's endothelium to resist that insult.[10]

In humans, CECs have been detected in diverse conditions of endothelial injury. Elevated levels were reported in various cardiovascular disorders, as a result of mechanical injury, ischemic injury or hypertension.[11,12]

Numbers of CECs were indicative of, and correlating with, the degree of endothelial injury.[13] They have both diagnostic and prognostic significance.[14]

Patients and Methods

The study was conducted on 50 subjects who were divided into 3 groups as follow:

Group I: Included twenty patients on conservative medical treatment from the out patients clinic or inpatient ward of the Nephrology unit Alexandria Main University Hospital, 7 were males and 13 were females. Their age ranged from 23 to 75 with a mean of 51.90 ± 14.69 years

Group II: Included twenty patients on maintenance haemodialysis three times weekly using bicarbonate as a buffer and polysulphone membrane, session duration and blood pump were adjusted to keep urea reduction ration ≥ 65%. Patients were selected from the haemodialysis unit Alexandria University Hospital, 9 were males and 11 were females. Their age ranged from 25 to 72 with a mean of 43.35 ± 13.08 years

Group III Included ten healthy subjects 4 were males and 6 were females. Their age ranged from 25 to 70 with a mean of 45.70 ± 13.90 years

Patients with the following criteria were excluded:

1. History of ischemic heart disease.
2. Diseases causing vasculitis e.g. SLE.
3. Patients on immunosuppressant or anti inflammatory therapy other than aspirin.
4. Diabetic patients.
5. Signs or symptoms of any clinical infection during the month previous to blood draw.
6. Active or past history of neoplastic or rheumatological diseases.
7. Hepatitis B or C infection
8. Central line insertion or any invasive procedure during the month prior to blood draw.

This study was conducted in accordance with the ethical standards of our Faculty of Medicine research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards

An informed consent was taken from all participants.

1. Peripheral Blood Sampling

Two ml of peripheral blood on K_3 EDTA were collected under complete aseptic conditions for complete blood count (CBC), morphological studies and determination of CECs (CD146) by flow cytometry. Another 5 ml was withdrawn for Renal functions, Complete lipid profile, ESR and High sensitivity CRP.

2. CBC and Morphological Studies

CBCs were performed on an automated cell counter Sysmex KX-21N; Serial number: A7291. Films were stained with Leishman stain, and were used for morphological identification of the differential count.[15]

Detection of Circulating Endothelial Cells

The detailed characterization of circulating endothelial cells is obtained by analyzing the expression of CD146 on mononuclear peripheral blood cells.[16]

In the present study, CD146 direct immunofluorescence technique was employed using PE labeled CD146 Abs.

Immunofluorescence on the leucocytes in suspension was analyzed using BD FACS caliber (Becton Dickinson) flow cytometer equipped with Cell Quest software.

Viable cells were incubated with the fluorescein labeled MoAb. Positive cells are then counted by the flow cytometer.

Statistical Methods

Data were analysed using SPSS software package version 18.0 (SPSS, Chicago, IL, USA). Quantitative data was expressed using Range, mean, standard deviation and median while Qualitative data were described using number and percent. Association between categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Firsher's Exact test. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test. D'Agstino test was used if there was a conflict between the two previous tests. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. For normally distributed Quantitative data was analysed using F-test (ANOVA) to compare the three categories of outcome. Not normally distributed quantitative data easanalysed using

Mann Whitney test for comparing two groups while for more than two groups Kruskal Wallis test was applied. Pearson coefficient was used to analyse correlation between any two variables. p value was assumed to be significant at 0.05.[17]

Results

Table (I) shows the lipid profile in the studied groups, the mean triglycerides level was significantly higher in groupI and group Π in comparison to the control group, Also the mean total cholesterol level was significantly higher in group I in comparison to the control group. Regarding the mean HDL it was significantly lower in group Π in comparison to group I and it was also significantly lower in the group I and Π

in comparison to the control group. There was no significant difference in the LDL level in between the three groups.

Table (II) shows serum ESR, CRP and CEC among the studied groups where the mean level of all variables was significantly higher in group I and group Π incomparison to the control group.

Circulating endothelial cell count was correlated positively with, TGs, CRP and ESR (Figure 1-3), while it was correlated negatively with GFR in CKD group and HDL in both groups.

There was a statistically negative correlations between CECs and serum albumin in group Π while there was negative correlation but statistically insignificant in group I.

Table (I): Comparison between different studied groups according to lipid profile.

	CKD (I)	HD (Π)	Control (Ш)	Test of sig
TG (mg/dl) Min. – Max. Mean ± SD Median	162.0 – 250.0 202.05±28.24 193.0	165.0 – 385.0 213.20±48.05 204.50	60.0 – 130.0 101.60±25.71 105.0	$^{KW}p<0.001^*$
p_1		$^{MW}p = 0.685$	$^{MW}p<0.001^*$	
p_2			$^{MW}p<0.001^*$	
Cholesterol(mg/dl) Min. – Max. Mean ± SD Median	138.0 – 203.0 174.80 ± 20.22 178.50	125.0 – 245.0 161.75 ± 33.13 146.0	130.0 – 170.0 154.80 ± 14.12 160.0	p = 0.098
p_1		$^{LSD}p = 0.111$	$^{LSD}p = 0.048^*$	
p_2			$^{LSD}p = 0.484$	
HDL(mg/dl) Min. – Max. Mean ± SD Median	38.0 – 49.0 42.55 ± 2.95 42.0	28.60 – 47.0 40.03 ± 3.85 40.0	40.0 – 55.0 47.90 ±4.53 48.0	p <0.001*
p_1		$^{LSD}p = 0.035^*$	$^{LSD}p<0.001^*$	
p_2			$^{LSD}p<0.001^*$	
LDL(mg/dl) Min. – Max. Mean ± SD Median	61.0 – 123.20 91.83 ± 19.56 96.10	43.0 – 169.0 80.84 ± 29.95 76.10	64.0 – 109.0 86.58 ± 12.40 89.0	p = 0.340
p_1		$^{LSD}p = 0.144$	$^{LSD}p = 0.565$	
p_2			$^{LSD}p = 0.529$	

p: p value for F test (ANOVA)
^{KW}p: p value for Kruskal Wallis test
^{MW}p: p value for Mann Whitney test
p : p value of Post Hoc test (LSD)
p_1 : p value between group CKD and each other group
p_2 : p value between group HD and group control

Table (2): Comparison between different studied groups according to ESR, CRP and CEC

|	CKD (I)	HD (Π)	Control (Ш)	Test of sig
ESR(mm/hr)1st hour Min. – Max. Mean ± SD Median	15.0 – 70.0 26.90 ± 15.80 21.50	20.0 – 90.0 34.85 ± 21.26 27.0	5.0 – 13.0 8.90 ± 2.51 9.0	KWp<0.001*
p₁		MWp = 0.067	MWp<0.001*	
p₂			MWp<0.001*	
CRP(mg/l) Min. – Max. Mean ± SD Median	3.0 – 19.0 6.95 ± 3.94 5.90	3.36 – 30.60 10.53 ± 7.41 9.21	2.97 – 2.97 2.97 ± 0.0 2.97	KWp<0.001*
p₁		MWp = 0.066	MWp<0.001*	
p₂			MWp<0.001*	
CECs(cells/ml) Min. – Max. Mean ± SD Median	6.30 – 59.94 26.13 ± 14.18 25.58	11.10 – 119.38 36.89 ± 24.60 31.50	3.0 – 17.0 9.76 ± 4.66 8.75	KWp =0.123
p₁		MWp = 0.123	MWp = 0.001*	
p₂			MWp<0.001*	

p: p value for F test (ANOVA)
KWp: p value for Kruskal Wallis test
MWp: p value for Mann Whitney test
p : p value of Post Hoc test (LSD)
p₁ : p value between group CKD and each other group
p₂ : p value between group HD and group control
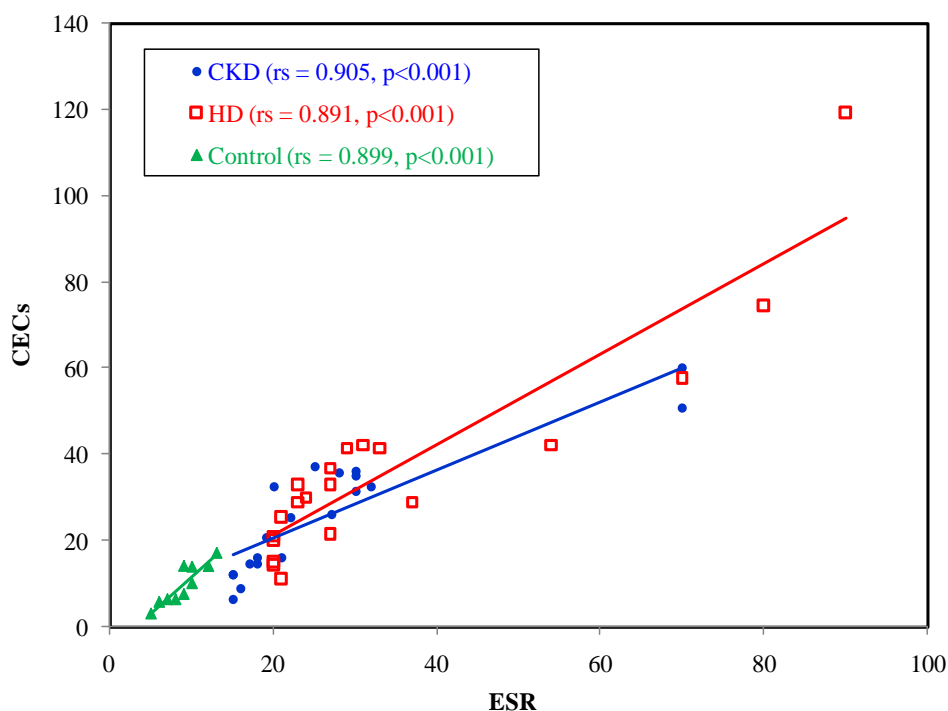
Figure 1: correlation between CEC and ESR in the studied groups.
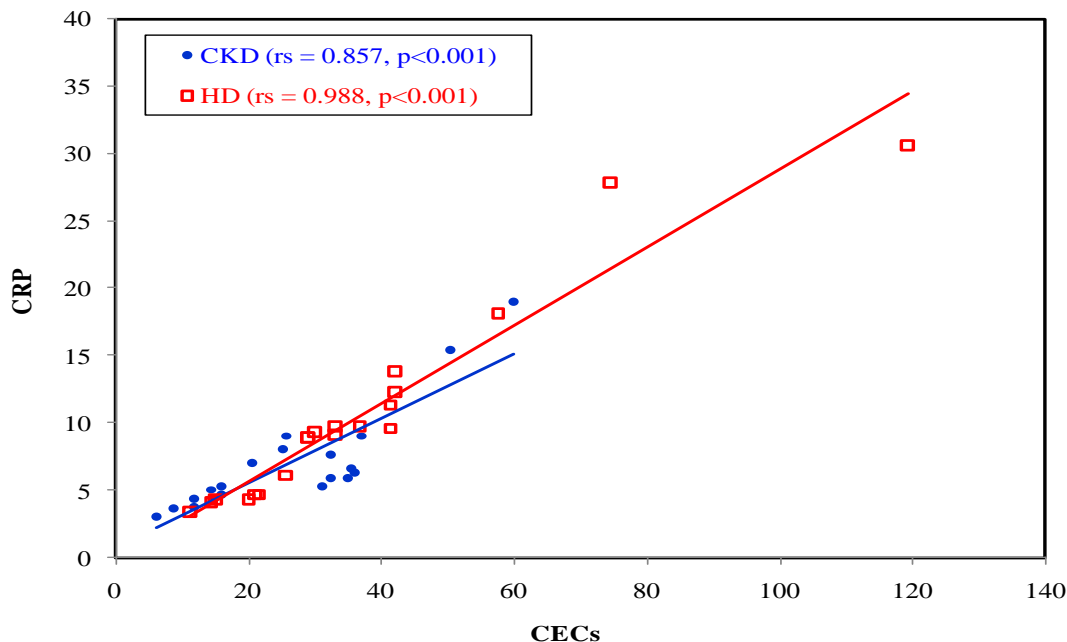
Figure 2: correlation between CEC and CRP in CKD and HD group
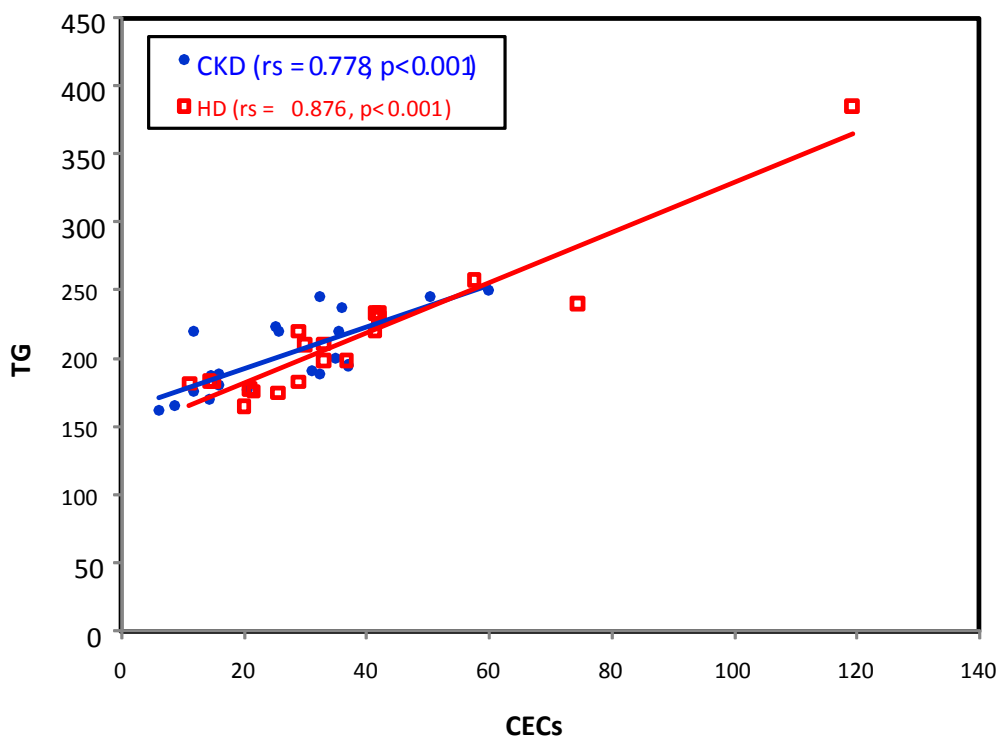
Figure (3): the correlation between CECs and TG in CKD and HD group

Discussion

Chronic kidney disease (CKD) is a risk factor for cardiovascular disease and is associated with increased all cause mortality. The increased risks are evident at even moderate reductions in kidney function. [18,19]

During the past few years, it has repeatedly been shown that the acute phase protein C-reactive protein (CRP) is a strong predictor of cardiovascular disease.[20]

Christoph Wanner et al,[21] confirmed that inflammation is associated with an increased

overall and cardiovascular mortality in hemodialysis patients A single determination of CRP is a powerful indicator of all cause and cardiovascular death even after a follow-up period of 4 years in patients on hemodialysis treatment. They concluded that prediction is even maintained after prolonged periods of observation.

So, elevated CRP in CKD and HD patients in this study may be an indicator that they are exposed to greater cardiovascular risk.

In comparing CECs count in CKD patients, ESRD patients on maintenance hemodialysis and normal subjects there was statistically significant higher levels of CECs in both CKD and ESRD patients in relation to the control group.

Detached circulating endothelial cell may serve as potential marker of endothelial damage in CKD.[22] CKD represents a condition with impaired migratory activity[23] and or decreased numbers[24,25]of circulating EPC , which may have a role in neovascularization of ischemic tissue and the progression of artherosclerosis and subsequent CVD.[26]

Imbalance between the expression of endothelial progenitor cells (reflecting endothelial repair capacity) and circulating endothelial cells (reflecting endothelial injury) seems to exist in CKD. this imbalance may contribute to the pathogenesis and progression of atherosclerosis process in CKD patients and may be linked to the ability of circulating inflammatory endothelial cells to interfere with functional capacity for vessel wall repair by EPCs.[27,28]

Mehmet et al[29] reported that in hemodialysis population, an increase in circulating endothelial cells was found to predict the development of cardiovascular and vascular events, and to be independent of other known markers of inflammation or endothelial dysfunction. This study is the first to report on the long-term follow-up of vascular events in hemodialysis patients after enumeration of CEC number. In this study, the mean CEC of healthy control patients has been used from the previous study to divide hemodialysis patients into two groups, patients

with "high" versus "low" number of CECs. In the ensuing 20 months, all 10 vascular events occurred in patients that were in the "high" CEC group despite the small number of patients in the this study, there is a statistically significant increased mortality and morbidity from vascular events in hemodialysis patients with a "high" number of CECs and a trend toward increased cardiovascular events.

Koc et al[21] asked whether or not CEC counts would be predictive of cardiovascular events in hemodialysis subjects at increased risk for CVD. They studied 2 patient cohorts: in the first, CEC numbers were determined in 29 hemodialysis patients followed up for a mean of 470 days; in the second cohort of 44 hemodialysis patients, they analyzed the association between CEC counts with other markers of vascular inflammation. Seven of the 19 subjects with elevated CECs (defined as >19 cells/ml) had cardiovascular events during follow-up, compared with no events among the low CEC count population (p = 0.04). Among the second cohort, there was a positive correlation between CEC counts and high-sensitivity C-reactive protein, interleukin-6, interleukin-10, monocyte chemoattractant protein-1, and soluble vascular cell adhesion molecule-1.

Also as a proof that CEC is indicator of vascular injury, George et al[30] plotted the effects of coronary angioplasty on CEC counts in 10 patients listed for elective coronary angioplasty. All patients had normal CEC counts before the procedure, with a peak increase in CEC counts to 8 to 15 cells/ml at 4 h after the procedure. There was then a gradual decrease in CEC counts over the ensuing 20 h toward near-baseline CEC levels. As number of CEC was indicative of, and correlating with the degree of endothelial injury [31] so, the marked vascular disease seen in hemodialysis and CKD patients is likely due to increased endothelial damage, represented by an elevated CEC number, as well as a decreased ability to repair the endothelial damage.

In the present study, there was a negative correlation between creatinine clearances and both CRP and CEC count so this means that the lower GFR carries a greater cardiovascular risk.

This coincides with what was mentioned by Paul Muntner et al[32] who showed that C-reactive protein levels were progressively higher at lower levels of estimated GFR.

In the present study, the level of CRP is significantly correlated with CECs count in both CKD and HD group with a P value of <0.001.

This is matching with the study done by Wang et al[33] where the investigators showed that CEC counts correlated with levels of inflammatory marker C-reactive protein, even after adjustment for confounding factors.

Conclusions

- CRP level is high in CKD patients which indicate the chronic inflammatory state they suffer from.
- Hemodilaysis patients had higher level of CRP that indicates they are at greater risk due to dialysis procedure.
- CECs count was found to be high in CKD and dialysis patients. .
- Measurement of CECs count might offer a mean for recognizing patients at risk of cardiovascular events.

Conflict of Interest: All authors declare that they have no conflict of interest.

References

1. Subramanian S, Tumlin J, *Bapat* B, Nikolsky E, Mehran R, Turcot DB, *et al.*Impact of *chronic kidney disease* on prognosis by the Society of Critical Care. Crit *Care* Med *2008;* 36:S204-S211.

2. Drey N, Roderick P, Mullee M, Rogerson M. A population based study of the incidence and outcomes of diagnosed chronic kidney disease. Am J Kidney Dis 2003; 42: 677–84.

3. McCullough PA. Cardiorenal risk: an important clinical intersection. Rev Cardiovasc Med 2002; 3:71– 6.

4. Pepys MB, Gill R, Kemp JA, Sabin C. CRP or not CRP? That is the question. ArteriosclerThrombVasc Biol. 2005; 25: 1091–4.

5. Arici M, Walls J. end stage renal disease, atherosclerosis and cardiovascular mortality: is C-reactive protein the missing link? Kidney Int 2001; 59: 407-14

6. Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. N Engl J Med 1990; 323:27-36.

7. Vane JR, Botting RM. Formation by the endothelium of prostacyclin, nitric oxide and endothelin. J Lipid Mediat 1993;6:395– 404.

8. Frick M, Weidinger F. Endothelial function: A surrogate endpoint in cardiovascular studies? Curr Pharm Des 2007; 13:1741–50.

9. Werner N , Kosiol S , Schiegl T ,Ahlers P ,Walenta K ,Link A et al . Circulating endothelial progenitor cells and cardiovascular outcome. N Engl J Med 2005; 353:999-1007.

10. Koc M, Bihorac A, Segal MS. Circulating endothelial cells as potential markers of the state of the endothelium in hemodialysis patients. Am J Kidney Dis 2003; 42:704-12.

11. Mutin M, Canavy I, Blann A, Bory M, Sampol J, Dignat-George F. Direct evidence of endothelial injury in acute myocardial infarction and unstable angina by demonstration of circulating endothelial cells. Blood 1999;93:2951-8.

12. Makin A , Chung NAY , Silverman SH , Blann A, Lip GY. Assessment of endothelial damage in atherosclerotic vascular disease by quantification of circulating endothelial cells. Eur Heart J 2004:25; 371-6.

13. Goon PKY Boos CJ, and Lip GYH. Circulating endothelial cells: markers of vascular dysfunction. Clin Lab 2005; 51, 531 – 8.

14. Furstenberger G , von Moos R , Lucas R , Thurlimann B, Senn HJ , Hamacher J , Boneberg EM. Circulating endothelial cells and angiogenic serum factors during neoadjuvant chemotherapy of primary breast cancer.Br J Cancer. 2006; 94:524 – 31.

15. Bain BJ, Lewis SM, Bates M. Basic hematological techniques. In: Bain BJ, Lewis SM, Bates I eds. Dacie and Lewis practical Hematology 10th ed. Philadelphia: Churchill Livingstone Elsevier 2006; 25-57.

16. Dignat-George F, Sampol J. Circulating endothelial cells in vascular disorders: new insights into an old concept. Eur J Haematol 2000; 65:215–20.

17. Leslie E, Geoffrey J, James M (eds). Statistical analysis. In: Interpretation and uses of medical statistics (4th ed). Oxford Scientific Publications (pub). 1991, pp.411-6.

18. Parfrey P. Is renal insufficiency an atherogenic state? Reflections on prevalence, incidence and risk. Am J Kidney Dis 2001; 37:154–7.

19. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med 2004; 351: 1296–305.

20. Owen FO, Lowrie EG, C reactive protein as an outcome predictor in maintenance haemodialysis patients Kidney Int 1998; 54:627-36.

21. ChristophWanner, Josef Zimmermann, Susanne Schwedler, Thomas Metzger. Inflammation and cardiovascular risk in dialysis patients Kid Int 2002; 61(Suppl 80): S99–S102.

22. Koc M, Bihorac A, Segal MS. Circulating endothelial cells as potential markers of the state of the endothelium in hemodialysis patients. Am J Kidney Dis 2003; 42:704-12.

23. HerbrigK ,Pistrosch F , Oelschlaegel U ,Wichmann G , Wagner A , Foerester S .increased total number but impaired migratory activity and adhesion of endothelial progenitor cells in patients on long term haemodialysis . *Am J Kidney Dis* 2004;44: 890-4.

24. Eizawa TMY, Matsui K, Takahashi M, Muroi K, Amemiya M, Takano R et al. circulating endothelial progenitor cells are reduced in haemodialysis patients .Curr Med Res Opin 2003;19: 627-33.

25. Choi JH, Kim KL, Huh W, Kim B, Byun J, Suh W et al .decreased number and impaired angiogenic function of endothelial progenitor cells in patients with chronic renal failure. Arterioscler ThrombVascBiol 2004; 24:1246-52.

26. Hill JM, Zalos G, Halcox JPJ, Schenke WH, Waclawiw MA, Quyyumi AA et al. circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 2003; 348: 593-600.

27. Rodriguez Ayala E , Yao Q, Holmen C , Lindholm B , Sumitran – Holgersson S , Stenvinkel P. embalance between detached circulating endothelial cells and endothelial progenitor cells in chronic kidney Disease . Blood Purif 2005;24:196-202.

28. Westerweel PE , Hoefer IE , Blankestijin PJ , De Bree P ,Groenveld D , Oostrom O et al . End stage renal disease cause embalance between endothelial and

smooth muscles progenitor cells .Am J Renal Physiol 2007; 292:F1132-F40.

29. Mehmet Koc, Hanno B. Richards, AzraBihorac, Edward A. Ross, Jesse D. Schold, and Mark S. Segal Circulating endothelial cells are associated with future vascular events in hemodialysis patients Kidney Int 2005; 67: 1078–83.

30. George F, Brisson C, Poncelet P, Laurent JC, Massot O, Arnoux D et al. Rapid isolation of human endothelial cells from whole blood using S-Endo1 monoclonal antibody coupled to immuno-magnetic beads: demonstration of endothelial injury after angioplasty. ThrombHaemost 1992; 67:147-53.

31. Goon PKY Boos CJ, and Lip GYH. Circulating endothelial cells: markers of vascular dysfunction. Clin Lab 2005; 51, 531 – 8.

32. Muntner P, He J, Hamm L, Loria C, Whelton PK. Renal insufficiency and subsequent death resulting from cardiovascular disease in the United States. J Am SocNephrol. 2002; 13:745–753.

33. Wang C, Li H, Fu P, Zhang S, Xiu R. Serum C-reactive protein and circulating endothelial cells in patients with acute myocardial infarction. ClinHemorheol Microcirc 2005; 32:287–96.