Study of the HCV Status Effect on Soluble P – Selectin Levels as a Marker of Platelet Activation in Hemodialysis Patients

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Abstract: In hemodialysis patients, soluble cellular adhesion molecule P- selectin has been found to be higher than normal. Chronic viral hepatitis C infection, highly prevalent in HD patients, is a disease that can induce chronic inflammation. Forty ESRD patients on prevalence hemodialysis were enrolled in the study; 20 HCV positive and 20 HCV negative patients. For all patients, the following was done: CBC, MPV, BT, blood urea, ALT, AST, ESR, CRP, total Cholesterol, and transferrin saturation percent. Serum soluble P – selectin as an indicator of *in – vivo* platelet activity, was measured by Immunosorbent assay (ELISA), with normal P – selectin range up to 100 ng / ml. We excluded from the study patients having liver cirrhosis, liver cell failure, DM, acute and chronic inflammatory states other than ESRD, HD, and chronic HCV infection, and lastly drugs affecting platelets. P-selectin levels were much above normal in the two studied groups with no significant difference between them.HCV positive patients showed significantly lower levels than HCV negative patients as regards WBC count ($P = 0.007$), platelet count ($P = 0.056$), total Cholesterol ($P < 0.001$). HCV positive patients showed significantly higher levels as regards MPV ($P = 0.05$), TSAT % ($P < 0.001$), ALT ($P = 0.031$), and AST ($P = 0.017$).We found a positive correlation between P–selectin and each of WBCs, blood urea, CRP, ALT, and AST, and an inverse correlation between P –selectin and serum albumin level.ALT, AST, WBC, MPV were the most important factors affecting P–selectin level in a direct relationship, while serum Albumin affected P -selectin level in an inverse manner. P–selectin is as much important as CRP, IF not more valuable in assessing the chronic inflammatory state induced by HCV infection in ESRD patients on prevalent HD.

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1. Introduction

Early stages of chronic kidney disease (CKD) are typically associated with a prothrombotic tendency, whereas in its more advanced stage patients also suffer from bleeding diathesis, suggesting that abnormal platelet function is a major contributor (*Van Bladel et al., 2012*).

Evidence suggests that the activation of platelets and their interaction with circulating cells are important independent risk factors for atherosclerosis in non-uraemic patients (Ashman et *al.,* 2003*).*

P – selectin (CD62) belongs to the selectin family of adhesion molecules. P–selectin is a biologically relevant molecule that is released to the surface of platelet from α granules on platelet activation and is found in a preformed state in Weibel – Palade bodies of endothelial cells and in α granules of platelets (*Cox, 1998; Chen et al., 2004; Scialla et al., 2011*).

Stored P-selectin is mobilized to the cell surface within minutes in response to a variety of inflammatory or thrombogenic agents, and is involved in the adhesion of myeloid cells to activated endothelium and in the adhesion of platelets to monocytes and neutrophils (*Chen et al.*, 2004).

Platelet P–selectin expression is increased during hemodialysis in association with increased platelet – leucocyte aggregation (*Ashman et al., 2003*). Moreover the soluble form is released into plasma during platelet activation independent of hemodialysis procedure (*Ozkan and Ulusoy, 2013*).

Hepatitis C virus (HCV) affects a large percentage of hemodialysis patients and may further aggravate their hemostatic abnormalities (*Pawlak et al., 2008*).

Reductions of platelet counts were more frequent in patients on dialysis particularly in the HCV positive patients. (Ando *et al., 2001*).

The use of erythropoietin (EPO) in CKD can enhance blood coagulation by stimulating production of E-selectin and P-selectin (*Vaziri and Zhou, 2009; Ribiero et al., 2013*).

There is evidence that a positive correlation between venous thrombosis, atherosclerotic cardiovascular disease (ASCVD) events and soluble P

selectin (sPsel) levels (*Ramacciotti et al., 2011; Scialla et al., 2011*).

2. Patients and Methods

Forty patients from different hemodialysis units in Cairo were enrolled in the study. One group comprised 20 HCV positive ESRD patients on prevalent hemodialysis and the other group comprised 20 HCV negative ESRD patients on prevalent hemodialysis. All patients included in the study were receiving 4 hours hemodialysis sessions three times weekly, using bicarbonate dialysate, low flux dialysers with surface area > 1.3 m^2 and Kt / V > 1.2, and using low molecular weight heparin as anticoagulant.All patients were above 18 years old, were receiving Erythropoietin (Epoetin Alpha) 2000 IU twice weekly, and were having transferrin saturation > 25 %. All patients were dialysed through native arterio-venous fistula (AVF).

We excluded from the study patients having liver cirrhosis, liver cell failure, DM, active inflammation (acute or chronic), autoimmune disease, allergic disease or receiving immunosuppressive drugs, statins or drugs affecting platelets. We also excluded patients with aplastic bone marrow disease, myeloproliferative disorders, and patients having co – existing HBV or HIV viruses.

All patients were subjected to complete physical examination. The following was done for all patients: Complete blood count (CBC) including platelet count (normal range 150,000 – 400,000 / mm^3) and mean platelet volume (MPV) in femtolitre (fl), with normal range from 7.4 to 10.4 fl (given that 1 femtolitre is equal to 1 cubic micrometer and using Automated Blood Counter to do it), Bleeding time using Ivy's method (BT > 9 minutes was considered as prolonged), serum creatinine in mg / dl(Yatzidis, 1974), blood urea in mg / dl by calorimetric method (Beal and Croft, 1961).ALT (normal range up to 33 U / L) and AST (normal range up to 31 U / L) using calorimetric method (Wilkinson et al., 1972), ESR in mm / hour, CRP in mg /L (normal range less than 8), total cholesterol (normal level < 180 mg / dl), and Transferrin saturation %.

Measuring soluble P- selectin by enzyme linked immunosorbent assay(ELISA) was performed for all patients enrolled in the study. P- selectin value in normal humans was considered to be 100 ng / ml (Polek *et al.,* 2009).Principle of the method included an anti –s P-selectin monoclonal coating antibody is adsorbed onto microwells. sP- selectin present in the sample or standard binds to antibodies adsorbed to the microwells; an HRP – conjugated monoclonal anti–sP-selectin antibody is added and binds to s P- selectin captured by the first antibody. A colored product is formed in proportion to the amount pf soluble P-selectin present in the sample or standard. A standard curve is prepared from seven s P- selectin sample concentration determined.Human P–Selectin ELISA KIT (www.bosterio.com): Boster Biological Technology Co., Ltd. 3942 B Valley Ave, Pleasanton, CA, 94566. (Boster Immunoleader).

Statistical Analysis

Data management and statistical analysis were performed using Statistical Package for Social Sciences (SPSS) vs. 21.for Windows. Continuous variables were analyzed as mean values ± standard deviation (SD) or median (range) as appropriate. Rates and proportions were calculated for categorical data. For categorical variables, differences were analyzed with χ^2 **(chi square)** tests.

3. Results

Table (1): COMPARISON AND ODDS RATIO BETWEEN HCV +VE And HCV - VE PATIENTS AS REGARDS AGE (Years), GENDER, DURATION OF DIALYSIS (Years), AND HCV INFECTION DURATION (Years).

	HCV(-ve)	**HCV(+ve)**		
Factors	**n=20(%)**	**n=20(%)**	**Test value**	***P* value**
Age (yrs)				
Mean± SD	50.7±15.6	46.1±12.4	t=1.029	0.310
Range	26-72	22-67		
Gender				
Male	10(50.0)	7(35.0)	χ^2=0.921	0.337
Female	10(50.0)	13(65.0)		
Duration of dialysis				
Median (range)	5(1-13)	7(1-15)	Z=-0.858	0.391
HCV duration				
Median (range)	NA	10(4-15)	NA	NA

NA = Not Applicable

Table (2): COMPARISON AND ODDS RATIO OF HCV + VE AND HCV –VE PATIENTS AS REGARDS COMPLETE BLOOD COUNT, ESR, CRP, BT (BLEEDING TIME IN MINUTES), AND TSAT (%).

	HCV(-ve)	HCV(+ve)			
Factors	**n=20(%)**	**n=20(%)**	**Test value**	**OR(95% CI)[a]**	***P* value**
	Mean± SD	**Mean± SD**			
Hb (mg/dl)	10.1±1.2	10.4±1.8	t=-0.840	1.2(0.8-1.8)	0.406
HCT	28.8±3.9	29.9±4.7	t=0.858	1.1(0.9-1.2)	0.396
WBC	8.3±2.2	6.4±2.3	t=2.827	0.6(0.5-0.9)	0.007
Platelet	259.0±84.0	202.7±95.8	t=1.971	0.9(0.8-1.0)	0.056
MPV	8.2±0.6	8.8±1.2	t=-2.027	2.2(0.9-5.4)	0.050
ESR(mm/h)	43.8±16.2	43.8±13.4	t=0.002	1.0(0.9-1.1)	0.998
CRP					
Median (range)	6.15(1-100)	7(3-90)	z=-0.271	1.0(0.9-1.1)	0.787
BT	5.0±0.1	5.3±1.1	t=-1.104	NA*	0.201
TSAT	25.1±3.1	34.9±11.0	t=-3.825	1.2(1.1-1.4)	<0.001

a: un adjusted OR (univariate level). OR: odds ratio, CI: confidence interval,
NA: not applicable
Regarding WBC with every one unit decrease in wbc count there is 40% risk to be HCV +ve

TABLE (3): COMPARISON AND ODDS RATIO BETWEEN HCV + VE AND HCV –VE PATIENTS AS REGARDS KIDNEY AND LIVER FUNCTION TEST.

	HCV(-ve)	HCV(+ve)			
Factors	**n=20(%)**	**n=20(%)**	**Test value**	**OR(95% CI)[a]**	***P* value**
	Mean± SD	**Mean± SD**			
Urea	147.2±48.3	141.1±39.2	t=0.442	0.99(0.98-1.1)	0.661
Creatinine	8.1±1.2	8.8±2.4	t=-1.212	1.2(0.8-1.7)	0.233
Albumin (g/dl)	3.6±0.3	3.5±0.3	t=1.108	0.3(0.1-2.4)	0.275
ALT	23.8±15.0	35.7±18.0	t=-2.247	1.1(1.0-1.3)	0.031
AST	19.8±12.1	30.9±15.8	t=-2.497	1.1(1.0-1.3)	0.017
S.Bilirubin	0.8±0.2	0.7±0.2	t=0.355	0.6(0.1-10.7)	0.724
Cholesterol	203.0±23.8	174.5±20.0	t =4.212	0.9(0.8-0.9)	<0.001

a: un adjusted OR (univarite level). OR: odds ratio, CI: confidence interval

TABLE (4): COMPARISON AND ODDS RATIO OF SERUM P – SELECTIN LEVELS (in ng / ml) BETWEEN HCV +VE AND HCV –VE PATIENTS

	HCV (-ve)		HCV (+ve)				
	Mean	**SD**	**Mean**	**SD**	**Test value**	**OR(95% CI)[a]**	***P* value**
p Selectin							
	1760.0	948.0	1910.0	787.0	t=-0.780	1.0(1.0-1.3)	0.589

a:un adjusted OR (univarite level). OR: odds ratio, CI: confidence interval

Table (5): CORRELATION OF P- SELECTIN TO DIFFERENT LABORATORY PARAMETERS IN ALL FORTY PATIENTS INVOLVED IN THE STUDY.

	P-selectin	
	r	*p*
Hb	-0.24	0.13
HCT	-0.16	0.3
Platelets	0.12	0.45
WBCs	0.33	0.035*
Urea	0.39	0.011*
CRP	0.47	0.002*
Albumin	-0.83	0.0001*
ALT	0.78	0.0001*
AST	0.69	0.0001*

This table shows a statistically significant direct correlation between p-selectin levels and WBCs, urea, CRP, AST and ALT and inverse correlation with serum albumin.

Table (6):MULTIVARIATE ANALYSIS FOR THE MOST IMPORTANT FACTORS AFFECTING P-SELECTIN LEVELS IN ALL FORTY PATIENTS INVOLVED IN THE STUDY.

	Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B	
	B	Std. Error	Beta	t	*P* value	Lower Bound	Upper Bound
(Constant)	5850.8	1425.8		4.103	<0.001	2961.7	8739.8
Albumin (g/dl)	-1252.5	362.7	-0.477	-3.453	0.001	-1987.5	-517.5
ALT	18.8	7.9	0.327	2.368	0.023	2.7	34.9

R^2=50.7- adjusted for WBCs-MPV-CRP-Urea

Table (7):MULTIVARIATE ANALYSIS FOR MOST IMPORTANT FACTORS AFFECTING P-SELECTIN LEVELS IN ALL FORTY PATIENTS INVOLVED IN THE STUDY.

	Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B	
	B	Std. Error	Beta	t	*P* value	Lower Bound	Upper Bound
(Constant)	-2358.8	1155.7		-2.0	0.049	-4702.6	-15.0
AST	23.9	7.6	0.415	3.1	0.004	8.4	39.4
WBC	139.9	45.8	0.389	3.1	0.004	47.1	232.7
MPV	300.0	124.7	0.332	2.4	0.021	47.1	552.9

R^2=50.3, adjusted for CRP-Urea

Kolmogorov-Smirnov test of normality was done to assess normality of continuous variables before starting the analysis. Differences among continuous variables with normal distribution were analyzed by **Student's T-test**; for continuous variables without normal distribution, we used non-parametric tests and differences were analyzed by the **Mann–Whitney U-test**. OR was calculated using logistic **regression analysis** was done. Dependent variable was HCV grouping

Pearson correlation was done to examine correlation between p selectin and different numeric variables.

Multiple linear regression analysis was done to determine variables that affect P-selectin level, only variables (WBCs, MPV, CRP, Urea, Albumin and AST) that were highly correlated with it were included in the model. Co-linearity was assessed and correlated covariates were excluded from the analysis

P value of $\leq$ 0.05 was considered statistically significant, *P* value of 0.056 to 0.09 was considered borderline significant *P* value $\leq$ 0.001 was considered highly significant, *P* value $\geq$ 0.1 is considered non – significant.

As regards multiple regression analysis model building, we hardly fulfilled assumption to do this analysis (as normality and equal variance). Sample size was a limiting factor to perform a good stong one.

4.Discussion

ESRD patients who are receiving Maintenance Hemodialysis are under the risk of increased oxidative stress. Oxidative stress has been previously speculated to be associated with activation of phagocyte oxidative metabolism by dialysis membranes, release of oxygen radicals during dialysis, direct peroxidation of lipids on the dialysis membranes, and exhaustion of antioxidant systems in uremic patients (*Locatelli et al., 2003; Tutal et al., 2010).*

It has been described that leukotrienes are generated during neutrophil – platelet interactions (Brady *et al.,* 1994), and that activated platelets induce superoxide anion release by monocytes and neutrophils through CD62P interaction (*Nagata et al.,*

1993; Cooper et al., 1994), which occurs during hemodialysis (unpublished data), (*Carreno et al., 1996).*

There have been a number of previous reports demonstrating both inflammation (Descamps - Latscha *et al.,* 1995; Haaber *et al.,* 1995;Bolton *et al.,* 2001; Stuveling *et al.,* 2003), and endothelial dysfunction (*Haaber et al., 1995 Mezzano et al., 1997; Thambyrajah et al., 2000; Bolton et al., 2001; Stuveling et al., 2003)*, among patients with CKD.

Raised circulating concentrations of markers of endothelial dysfunction, such as von Willebrand factor (vWf),(*Blann and Mccollum, 1999; Whincup et al., 2002; Landray et al., 2004),* and soluble Pselectin (sPsel) (*Blann et al., 1997; Ridker et al., 2001, Landray et al., 2004)*may be associated with an increased risk of cardiovascular events.

In a published systematic review related to HCV infection in Eastern Mediterranean Regional Office of WHO, the prevalence rate of HCV infection in hemodialysis (HD) patients in Egypt was 48 % and it is the most common cause of chronic liver disease (*Alavian et al., 2012).*

HCV infection has been noted to be associated with increases in serum inflammatory cytokines (*Kalantar - Zadeh et al., 2005).* There have been suggestions that HCV infection is associated with markers of malnutrition – inflammation complex syndrome MICS in the MHD population, but this area was largely unexplored (*Kalantar – Zadeh et al., 2004).*

Tardif et al., 2005; *Sezer et al.*, 2006; and *Tutal et al., 2010*; in their studies had contradictory results, reporting HCV infection as both a cause and a possible protector of oxidative stress.

We didn`t find any significant difference in age and gender between HCV negative and positive patients, but we noticed that female gender represented a greater proportion of the patients in HCV positive HD patients, in a statistically non – significant way. This means that our results were not affected by difference in the range of age or gender distribution.

Duration of dialysis was longer in HCV positive HD patients in a non – significant way.

Patients with renal disease have been at increased risk of acquiring HCV because of prolonged vascular access as well as the potential for exposure to infected patients and contaminated equipment *(Abo Seif et al., 2012).*

Although HCV infection duration median was 10 years, we didn`t have any patients having cirrhosis or liver cell failure, (Child' s B & C stages).

Both HCV positive and negative HD patients had serum soluble P – selectin levels very much higher than normal value in healthy individuals. Mean value of serum soluble P – selectin was slightly higher in HCV positive HD patients, in a non – significant way (P = 0.589).This could be due to the lesser effect of HCV infection in the presence of uremic state of ESRD, on different blood cells and their cytokines and chemokines mediating inflammatory responses.But the slight increase in P –selectin level, as an adhesion molecule, in HCV positive patients reveals that, on the long run, HCV infection is not as benign as it appears.

Soluble P-selectin (sP-selectin) has been proposed as a marker of *in vivo* platelet activation (*Blann and Lip, 1997; Ferroni et al., 2001).*

Increased levels of some soluble adhesion molecules have been found in patients with impaired renal function (*Bonomini et al., 1998; Peng et al., 2005)* and in hemodialysis patients as compared to healthy controls (*Fadel et al., 2014).* In this study, soluble P-selectin positively correlated with the erythropoietin (EPO) dose in hemodialysis children, and a significant positive association was found between

Soluble P-selectin with thrombosed AVFs, (r = 0.83, P=0.04).

Many stimuli leading to increase in the expression of P-selectin can be present in patients on HD as a result of blood exposure to the artificial surfaces (*Bonomini et al., 1998; Abou – Shousha and Youssef, 2006).*

Carreno et al, 1996, observed that, whatever the membrane used, all patients had high levels of circulating soluble P – selectin before starting the dialysis procedure as compared to healthy individuals. The levels were not affected during the dialysis session.

Stasko et al., 2007. found that the predialysis soluble P –selectin plasma levels did not differ significantly compared with those of the healthy controls, but there was a significant increase of soluble P – selectin levels after a single HD session, in HD patients receiving and not receiving Erythropoietin supplement.

On the contrary, in the study of *Bossola et al., 2012,* P-selectin showed a transient decrease over time, and the serum levels of these molecules were not associated with CVD or with CVD-related mortality.

In vivo platelet activation occurs in patients with chronic C hepatitis and seems to be related to the severity of disease(*Ferroni et al., 2001).*

Plasma sP-selectin levels were higher in hepatitis C patients compared with normal controls (P<0.0001), (*Ferroni et al., 2001).*

Bleeding time in both HCV positive and negative HD patients was within normal limit for the method used, although it was slightly longer in HCV positive patients in a non – significant way (P = 0.201)

Although ESR level has been higher than normal in both HCV positive and negative groups HD patients, the difference between them was minimal and insignificant, which may imply that against what was expected, the participation of HCV infection chronic inflammatory state by itself had a minimal, if any participation at all in ESR rise above normal in HCV positive HD patients. This also means that chronic inflammatory state created by ESRD and Hemodialysis took the upper hand in both groups, ESR being only slightly (in a non – significant way), higher in HCV negative patients on exogenous Erythropoietin supply only as compared to HCV positive patients having both endogenous and exogenous Erythropoietin supply, as Erythropoietin has an anti – inflammatory effect counteracting factors causing chronic inflammatory states existing in both HCV positive and negative HD patients.

CRP median levels were within normal range in both groups, although it was slightly higher in a non – significant way in HCV positive patients groups. This again supports the idea that HCV infection in Hemodialysis patients doesn t have much more inflammatory impact than does Hemodialysis alone for ESRD without HCV infection.

Sezer et al., 2006; and Tutal et al., 2010, previously reported that chronic HCV infection in HD patients was associated with decreased plasma levels of oxidative stress indicators and a tendency toward increased plasma antioxidative capacity. *Sezer et al. 2006,* have deduced that HCV infection in dialysis patients may impair oxidative agent synthesis.

On the contrary, *Lieber, 1997; Paradis et al., 1997; Larrea et al., 1998; Okuda et al., 2002; Mahmood et al., 2004; and Tardif et al., 2005;* stated that HCV infection is associated with increased oxidative stress both in the normal and the dialysis population.

CRP has shown a positive correlation with serum soluble P – selectin in all forty patients (P = 0.002), although we had CRP levels within normal in both HCV positive and negative HD patients, and very high levels of serum soluble P – selectin adhesion molecule in these two groups. CRP relationship to serum soluble P – selectin was not confirmed in Multivariate Analysis, as CRP was not one of the most important factors affecting P – selectin, which means that CRP doesn ‘t have a direct impact on serum soluble P-selectin level. This may decrease the importance of CRP as an indicator of chronic inflammatory state present in dialyzed ESRD patients, and for a much more extent as an inflammatory marker for HCV infected hemodialyzed ESRD patients.

While Hb level in the two studied groups was less than recommended 11 gm / dl, Hb and HCT were slightly higher in HCV positive patients in a statistically non – significant way, may be due to endogenous Erythropoietin secreted within HCV positive patients, together with exogenous supply used for all studied patients. May be for the same reason, MPV was significantly higher in HCV positive patients group (P = 0.05), with an OR of 2.2 (95 % CI 0.9 – 5.4).

Sabry et al., 2007, reported that HCV-positive and HCV-negative Egyptian chronic hemodialysis patients have comparable hemoglobin as well as hematocrit levels.

Elsaran et al., 2009, in their study showed that ESRD patients on HD with HCV infection had significantly higher Hb and HCT levels compared with HCV-negative patients.

Farag et al., 2012, in their study found that higher hemoglobin levels were associated with increased P- selectin levels in patients on ESA treatment, but not in ESA-naïve CKD patients, suggesting a potential influence of ESAs on platelet activation when targeted for higher hemoglobin levels.

While being within normal range in the two studied groups, Inspite of endogenous and exogenous Erythropoietin supply, White blood cell count was much significantly lower in HCV positive patients group (P = 0.007), with an OR of 0.6 (95 % CI of 0.5 – 0.9).This may be due to margination of white blood cells or their migration outside the intravascular compartment, as we had high P – selectin levels which could be due to increased platelets - leukocytes interaction.

Stokes and Granger, 2012, demonstrated the “ *Cross - Talk* “ between platelets, vascular endothelium, and leukocytes in response to stimuli released during inflammatory condition.

Cell adhesion processes are activated during hemodialysis procedure and are able to initiate the leukocyte inflammatory response, that itself may play an important role in the adverse effects of hemodialysis. Peripheral leukopenia during the early phase of hemodialysis is due in part to an accumulation of leukocytes in the lung vasculature (*Toren et al., 1970; Dodd et al., 1983; Schaefer et al.; 1990; Carreno et al., 1996).* It has been described that during inflammation, adhesion molecules are involved in cell margination, activation, and subsequent transendothelial migration (*Gorsky, 1994; Brady, 1994; Carreno et al., 1996)*

Platelet count which was within low normal limit, was also lower in HCV positive patients group in a borderline significant way (P = 0.056), with an OR of 0.9 (95 % CI of 0.8 – 1.0).MPV was within low normal range, being significantly higher in HCV positive patients (P – 0.05), may be due to the additive effects of endogenous and exogenous erythropoietin, or may be this may be correlated to the slightly higher

soluble P – selectin and CRP levels in HCV positive patients that could indicate the presence of a greater degree of inflammation in these patients, due to the superadded effects of ESRD, HD, and chronic hepatitis C inflammatory state. In all forty patients involved in the study, we found a positive correlation between White blood cells count and serum soluble P – selectin level ($P = 0.035$), MPV and serum soluble P – selectin level ($P = 0.021$). Multivariate Analysis confirmed these relationships, as White blood cell count and MPV were among most important items having an impact upon serum soluble P – selectin levels in a direct relationship pattern.

Abd El – Azeem et al., 2012, in their study found that the mean platelet count was significantly higher in HD patients having a history of thrombosis when compared to HD patients having no previous events. In the same study, the mean platelet volume for all studied patients together (with and without thrombotic events), was within normal range..

Hemodialysis is also associated with P–selectin (CD62P) / sialyl – Lewisx (CD 15s) interactions which mediate platelet – leukocyte coaggregation (*Weksler, 1983; Carreno et al., 1996).*

Adhesion of activated platelets to leukocytes, which is transient and depends on transient expression of selectins shed into circulation by activated cells, may induce a reciprocal cell activation which could mediate cellular damage to the endothelium (*Hakim and Schafer, 1985; Larsen et al., 1989; Gamble et al., 1990; Henson, 1990; Rinder et al., 1991; Faint et al., 1992; Gearing and Newman, 1993).*

Among numerous complications, patients with HCV infection may develop abnormalities in peripheral cell counts such as anemia, neutropenia, and thrombocytopenia (*Anwar et al., 2011).*

Mild-to-moderate thrombocytopenia and platelet functional abnormalities have been described in patients with chronic hepatitis (*Joist et al., 1994; Ferroni et al., 2001; Giannini and Savarino, 2010).* In particular, a defective primary hemostasis (hemostatic platelet plug formation) in the absence of thrombocytopenia or out of proportion to the extent of thrombocytopenia was observed that seemed to correlate with the presence of abnormal platelet aggregation and severity of liver disease (*Rubin et al., 1979; Ferroni et al., 2001).*The platelet functional defect seems to be intrinsic and not solely a result of indirect *mechanisms (Laffi et al., 1988; Laffi et al., 1992; Ferroni et al., 2001).*

Beyan, 2012, stated that it was not proven that MPV is a marker for platelet function.

Alterations in platelet function is observed in patients with chronic uremia including decreased sensitivity to platelet agonists *(Alves Rios et al., 2010; Van Bladel et al., 2012)*, adhesion to strange surfaces,and reduced procoagulant activity (*Alves Rios et al., 2010).*

Formation of platelet microaggregates in the blood of HD patients may also contribute to the low platelet count. Decrease in MPV during Hemodialysis is probably due to the formation and deaggregation of microaggregates.Density and volume of platelets deaggregated are lower than normal platelets (*Cieslar et al., 1979; Ozdemir et al., 1997).*

Schoorl et al., 2008; El – shamaa et al., 2009; Schoorl et al., 2011), reported that due to the ongoing platelet activation in subjects on maintenance HD, platelet characteristics in peripheral blood show increased amounts of depleted platelets with a smaller volume and a shortened life span.

On the contrary, *Tanaka et al., 1989; and Khuri et al., 1992,* stated that MPV was found to be increased in maintenance HD because of rapid platelet turnover and formation of young platelets.

TSAT was much significantly higher in HCV positive patients group ($P < 0.001$), with an OR value of 1.2(95% CI 1.1 – 1.4).TSAT is a negative phase reactant, and this strange result may be due to interference of endogenous and exogenous erythropoietin present in HCV positive patients.

Dialysis efficiency and nutritional state didn‘t show any significant difference between HCV positive and HCV negative patients groups.

Serum creatinine was slightly lower in HCV negative HD patients as compared to HCV positive patients, in a non – significant way, while blood urea was slightly higher in HCV negative HD patients as compared to HCV positive patients, in a non – significant way.

Sezer et al., 2005, reported that predialysis serum creatinine levels were lower in HCV positive HD patients than HCV negative patients.

Albumin mean levels were within normal in both groups and minimally higher in HCV negative patients, in a non – significant way.

Sezer et al., 2006; and Tutal et al., 2010, found that albumin levels were lower in HCV positive than HCV negative HD patients, so they have speculated that hypoalbuminemia, as a negative acute phase reactant, may due to increased inflammation in infected patients rather than hepatic dysfunction.

An inverse correlation existed between serum albumin and serum soluble P –selectin level (P=0.0001). This was confirmed by multivariate analysis showing serum albumin as one of the most important factors affecting serum soluble P –selectin level in an inverse relationship pattern.

Although ALT level was just above normal range limit in HCV positive HD patients, ALT liver enzyme mean value was much significantly higher in HCV positive HD patients than HCV negative HD

patients (P = 0.031), with an OR value of 1.1 (95 % CI 1.0 – 1.3), inspite that these patients group didn ' t show higher ESR or CRP levels than HCV negative hemodialysis patients, which may reflect a subtle inflammatory state.

Vendemiale et al., 2001, and Tutal et al., 2010, in their study also indicated that HCV carriers with persistently normal ALT levels may show oxidative alterations in the absence of other clear signs of disease.

ALT levels were normal and similar between anti-HCV-positive and HCV -negative HD patients, in the study conducted by *Tutal et al., 2010.*

Although AST level was at high normal range limit in HCV positive HD patients, AST was much significantly higher in the HCV positive group (*P* = 0.017) with an OR value of 1.1 (95 % CI 1.0 – 1.3) and this confirms the subtle inflammatory state existing within HCV positive HD patients.In all forty patients we found a positive correlation between each of ALT and AST and serum soluble P –selectin levels (*P*= 0.023 and *P*=0.004, respectively).Multivariate Analysis has confirmed these two items among the most important factors affecting serum soluble P–selectin levels in a direct relationship pattern.This could lead us to deduce the importance of the adhesion molecule soluble P–selectin as a more dependable marker of subtle inflammatory state, which was not severe enough in both groups, despite chronic inflammatory state of ESRD, Hemodialysis, and HCV infection to cause a substantial rise of CRP.

Sezer et al., 2006; and Tutal et al., 2010, findings have made them believe that it was not the presence of HCV infection but the activity of the disease that caused increased oxidative stress among MHD patients. Also, it seemed that inactive HCV infection is a protective condition against increased oxidative stress in MHD patients.

Serum Bilirubin was minimally lower in HCV positive HD patients in a non significant way.

Alsaran et al. 2009, found that on comparing HCV positive and negative HD patients, liver function tests were normal except for higher bilirubin level in the HCV-positive group (*P* = 0.01).

While HCV positive total cholesterol level was at the high normal range limit, may be due to dyslipidemia present in ESRD. Total cholesterol was much significantly less in HCV positive HD patients (*P* < 0.001), with an OR value of 0.9 (95 % CI of 0.8 – 0.9). This doesn ' t mean that HCV infected HD patients have less dyslipidemic state than non – infected HD patients of the same range of age and nearly the same gender distribution, but may be due to the presence of the less ability of HCV infected liver cells to synthesize total cholesterol, which is in accordance with the significantly higher ALT and AST liver enzymes in HCV positive HD patients.

Sezer et al., 2006, reported that HCV infected dialysis patients showed decreased total cholesterol level as compared to HCV negative HD patients.

Ferroni et al., 2001, found total cholesterol levels were lower in chronic hepatitis C patients compared with healthy subjects (P < *0.005).*

Conclusion

Serum soluble P – selectin is a good indicator of in vivo platelet activation and should be used in HCV positive HD patients among the follow up laboratory tests set for follow up of these patients, as it can, together with different liver function tests, be a marker of endothelial dysfunction and subtle inflammatory state apparently masked by ESRD manifestations in these patients.

For future research to focus on SERUM ALBUMIN, AST, WBCs, MPV and CRP as regards their relationship to SERUM P – SELECTIN, but this should be conducted with a larger sample size.

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