ORIGINAL ARTICLE
Evaluation of CD markers (5, 19, 56) in HCV patients Undergoing Hemodialysis

[1]*Sherif.M. EL-Sherbini*, [2]*Fatma.E.Osman*, [3]*Samir.A.EL-Masry*, [4]*Abdelhakem S. Eldeen*

Department of Molecular Biology, Genetic Engineering and biotechnology Institute,
EL-Sadat University- El-Sadat City, Egypt
[1]Lecturer of Immunology, Institute of Genetics Engineering and Biotechnology, University of Sadat City
[2]Mediacal Analysis Specialist, Cairo, B.Sc. Chemistry-Zoology, Faculty of Science, Ain-shams University
[3]Professor of Biochemistry, Institute of Genetics Engineering and Biotechnology, University of Sadat City
[4]Professor of Immunology, Faculty of Science, Cairo University

ABSTRACT

Key words:

Chronic hepatitis C-end stage renal disease- immune response

Background*: The profile of the immune response of B- lymphocytes markers; CD19^{+} CD5^{+} and NK cells CD56^{+} in chronic hepatitis C and end-stage renal disease patients on Hemodialysis is still limited.* ***Objective****: to study the peripheral bloo immunophenotypic features of NK, B1 and B cells among patients with Hepatitis C virus infection undergoing hemodialysis, compared to normal patients, by three color flow cytometry immunophenotyping.* ***Methodology****: Immunophenotypic features of peripheral blood leucocytes were assessed by flow cytometry in two distinct groups: HCV patients with ESRD (CHC+ESRD, n = 15) and HCV patients with normal renal function (CHC, n = 20), Two control groups that were included were as follows: healthy individuals served as a control group with negative HCV-RNA–PCR (Control, n=15) and patients on hemodialysis with negative HCV-RNA –PCR (ESRD, n=20).* ***Results:*** *Flow Cytometry detection of B-lymphocytes and NK-cells showed that, the percentage of B lymphocytes (CD19^{+}) were significantly increased in (CHC+ESRD) group compared with ESRD group and normal group (P<0.05), NK cells (CD56^{+}) decreased in (CHC) group compared with (CHC+ESRD) group and control group but it was not a significant decrease. Also, (CD56^{+}) decreased in (CHC+ESRD) group compared with ESRD control group but without a significant P value (P >0.05). The B1 lymphocytes (CD5^{+}) were decreased in (CHC) group compared with (CHC+ESRD) group and control subjects, There was a slightly decrease in (CHC+ESRD) group compared with ESRD control group, No statistically significant difference was shown between groups (P>0.05).* ***Conclusions****: Chronic hepatitis C patients with ESRD on HD show distinctive phenotypic profile of circulating leucocytes. It may be a result of HCV infection in this particular group of patients and further investigation on larger scale of patients is recommended.*

INTRODUCTION

Hepatitis C virus (HCV) is a globally prevalent pathogen and a leading cause of death and morbidity[1]. Persistent HCV infection is associated with the development of liver cirrhosis, hepatocellular cancer, liver failure, and death [2].

HCV infection is a major public health issue, which affects approximately 2.8% of the world's population[3,4]. Egypt has the highest prevalence of hepatitis C virus (HCV) in the world, estimated nationally by 14.7%, with genotype 4 being the most common [5].

Transmission occurs mainly via blood contact. Hence, the prevalence of HCV infection is significantly higher in hemodialysis and, consequently, in kidney transplant recipients than in the general population, positively correlated with a history of multiple blood transfusions and time on hemodialysis. Since the discovery of HCV in 1989 and the subsequent beginning of screening for HCV in the early 1990s, incidence rates of HCV infection have dropped.[6]

However, in developing countries with high prevalence of HCV, nosocomial transmission through transfusions, hemodialysis or unsafe injections is still an important issue[7].

*Corresponding Author:
Sherif el-sherbini,
Institute of Genetics Engineering and Biotechnology,
University of Sadat City
E-mail: firstsherif_2000@yahoo.com; Tel :01066886718 0502318064

End stage renal disease (ESRD) is associated with both inflammation and immune deficiency [8,9,10.]

It is now known that altered T lymphocyte function, found in ESRD, can be attributed to impaired function of Antigen presenting cells (APCs)[11]. Because T-cell activation by APCs is dependent to a great extent on TLRs, ESRD, and especially HD, is associated with B-cell lymphopenia. It has been suggested that one of the major causes of this disturbance is an increased susceptibility to the death of B cells by apoptosis [12].

The immune system plays an important role in hepatitis C virus (HCV) infection. The pathogenic process of chronic hepatitis C (CHC) is still not fully understood. Previous studies have shown that poor T cell immunity is associated with the pathogenesis of CHC [13]. However, the regulation of T cell immunity against HCV during the process of CHC has not been fully understood. As our knowledge about the immunological aspects of the chronic hepatitis C (CHC), especially in patients with end-stage renal disease (ESRD), is still narrow. The course of HCV infection, the most important cause of chronic liver disease in ESRD patients on regular hemodialysis (HD), has unusual pathogenesis and natural history [14, 15].

There is a shortage of studies objecting the correlation of viral clearance and the immune status of patients with CHC and ESRD. As CHC has a wide range of clinical manifestations, varying from minimal hepatic lesions to cirrhosis, the factors affecting the origin and severity of disease progression need to be studied. Distinct clinical and histological presentations of CHC have suggested an interaction of multiple viral and host factors and the combination of different immunologic parameters to explain the hepatic injury caused by HCV infection in ESRD patients. Hence, the better comprehension of the immune response patterns may help to explain the natural history of HCV infection and the diversity of clinical presentations of this particular group of patients [16,17].

Evaluation of different populations of lymphocytes, namely T lymphocytes (including T helper and T cytotoxic cells), B lymphocytes, and natural killer (NK) cells, is an effective method to evaluate the integrity of an individual's cellular and humoral immunity[18,19]

The immunophenotyping of peripheral blood has been employed in studies focusing on the pathogenesis of CHC. A correlation among some immunophenotypic profiles, have been suggested recently [20,21]. Hence, we investigated the profile of the immune response in CHC patients with ESRD on HD, focusing on the major phenotypic features of peripheral blood lymphocytes.

METHODOLGY

1-Study group

Thirty five patients were enrolled, the groups and inclusion/exclusion criteria were established as follows: (a) patients with end stage renal disease on hemodialysis and chronic HCV infection (n= 15) (CHC+ESRD group); and, (b) treatment naïve uncomplicated chronic hepatitis C patients   (n = 20) anti-HCV and HCV-RNA (AMPLICOR, Roche Molecular Systems, lower limit of detection of 16 IU/ml) positive, with normal renal function (CHC   group). Exclusion criteria were decompensated cirrhosis, HBV and/or HIV co-infection, concomitant autoimmune disease, alcohol intake and use of immunosuppressive drugs. Control groups were as follows: (a) voluntary Egyptian blood donors (Control group) (n= 15), (b) ESRD patients on HD with anti-HCV negative (3rd generation ELISA), normal ALT, negative HCV RNA-PCR (ESRD group) (n= 20). table 2 shows the characteristics of the patients groups.

2-Laboratory proceedings:

Peripheral blood samples were collected from all participants and promptly analyzed. EDTA-treated peripheral blood white cells were labelled with fluorochrome conjugated specific monoclonal antibodies following immunofluorescence analysis by flow cytometry. Qualitative and quantitative tests (PCR, AMPLICOR, Roche Molecular Systems Inc., US kits) were used to confirm diagnosis of HCV infection and measure of HCV viral load.

a-Immunophenotyping of peripheral blood leucocytes:

White blood cell phenotypes were analysed 'ex-vivo', following an immunofluorescence procedure recommended by Beckman coulter kit. Briefly, 50 µl aliquots of EDTA whole peripheral blood samples were mixed in 12 x 75 mm tubes with 5 µl of undiluted monoclonal antibodies (mAbs) specific for several cell-surface markers labeled with fluorochrome (fluorescein isothiocyanate, phycoerythrin and tricolour). The list of mAbs used in this study is provided in (Table 1). The tubes were incubated in the dark for 15 min at room temperature. Following incubation, erythrocytes were lysed using 1.5 ml of fluorescence activated cell sorter (FACS) Lysing Solution. After incubation, the cells were washed twice with 2 ml of phosphate-buffered saline containing 0.01% sodium azide. Cell preparations were fixed in 200 µl of FACS fix solution (10 g/L paraformaldehyde, 1% sodium cacodylate, 6.65 g/L sodium chloride, 0.01%sodium azide). Cytofluorimetric data acquisition was performed with a Cytofluorimetric data acquisition was performed with a coulter Epics XL-USA instrument.

b-The immunophenotyping covered the followings:

Cellular populations and surface markers: Frequency of leucocyte subsets; Natural Killer (NK) cells (CD56) and B cells (CD19, CD5).

Table 1:-Monoclonal antibodies (mAbs) used for immunophenotypic analysis.

mAbs	Fluorochrome	Clone	Target
Anti-CD5-FITC	FITC*	BL 1a	B1-cells
Anti-CD19-PC5	PC5**	J4,119	B- cell
Anti-CD56-PE	PE***	N901 (NKH-1)	NK-cells

*FITC , fluorescein isothiocyanate; **PC, R phycoerythrincyanin5,1; ***PE, R phycoerythrin

3-Statistical analysis:

All statistical analysis were performed using SPSS software (version 23.0, SPSS), Non-parametric ANOVA was used to detect statistical differences in mean percentages and counts of B lymphocytes subpopulations and NK-cells. P value <0.05 was considered as significant. All calculations were performed using the Statistical Package for Social Sciences version 23.0 software.

RESULTS

Table 2:- Demographic characteristics of patients.

Parameter	CHC+ESRD group	CHC group	ESRD group	Control group	P value
Number (N)	15	20	20	15	--
Gender (♂:♀)	7/8	10/10	8/12	5/10	--
Age (Years)	53.7±13.39	49.9±11.85	49.5±13.51	35.9±14.95	P<0.01

Biochemical and clinical characteristic of patients versus healthy control shows that both AST and ALT were increased by HCV infection (CHC+ESRD group & CHC group) compared with ESRD control group and normal control group, there were a statistically Significant difference in AST (P<0.01) and ALT (P<0.001) of different groups of patients when compared with control groups, a significant difference between patients groups and control groups was found in total bilirubin , Alkaline phosphatase and Albumin (p<0.001), (P<0.01) and (P<0.001) respectively.

Blood urea and serum creatinine were increased by ESRD (CHC+ESRD group & ESRD group) compared with (CHC group & control group), there were a statistically significant between the different groups and normal control , Urea (P<0.001) and creatinine (P<0.001) as shown in table 3.

Table 3:- Biochemical and clinical characteristic of patients versus healthy control

Parameter	CHC+ESRD group	CHC group	ESRD group	Control group	P value
AST (U/l)	37.6±15.56	36.50±13.39	27.60±6.93	24.93±5.68	P<0.01
ALT (U/l)	37.46±14.98	33.00±11.07	22.60±5.01	22.93±5.53	P<0.001
T.Bil. (mg/dl)	0.68±0.11	0.79±0.11	0.61±0.09	0.68±0.07	P<0.001
ALP (U/l)	153.80±37.01	177.00±31.59	194.70±28.92	160±42.97	P<0.01
Alb (g/L)	3.48±0.35	3.55±0.40	3.64±0.26	4.08±0.20	P<0.001
Urea (mg/dl)	117.53±33.28	29.55±3.76	123.85±26.87	26.40±5.60	P<0.001
Creatinine (mg/dl)	9.07±2.12	0.93±0.13	9.25±1.77	0.80±0.16	P<0.001

All data were presented as mean± SD. Chronic HCV (CHC),end stage renal disease (ESRD), Alinine amoinotransferase (ALT),Aspartate aminotransferase (AST), Total Bilirubin (T.Bil.), Alkaline phosphatase (ALP), Albumin (Alb).

Hematological characteristic of patients versus control groups showed that ESRD patients on HD had a significantly decreased in mean of hemoglobin concentration (P<0.01) compared to control subjects, while total Leucocytes counts and platelets counts were not significant between groups of patients compared with control subjects (P>0.05). Total Lymphocytes counts were decreased in (CHC, ESRD & CHC+ESRD) groups compared with control group. Also it, increase in (CHC+ESRD) group compared with CHC group and ESRD. Total lymphocytes were significant (P<0.05) between groups of patients compared with control subjects. as shown in table 4.

Table 4:- Hematological characteristic of patients versus control groups:

Parameter	CHC+ESRD group	CHC group	ESRD group	Control group	P value
HB (g/dl)	10.42±1.61	11.74±1.36	9.78±1.86	11.04±0.90	P<0.01
WBCs (Thousand/cmm)	7.26±4.00	5.37±1.76	7.26±1.90	6.32±1.61	N.S
LYM (%)	24.28±7.41	23.09±9.66	18.80±4.47	26.97±8.59	P<0.05
PLT (Thousand/cmm)	158.93±44.87	244.90±44.91	255.00±54.60	270.33±56.18	N.S

Hematological characteristic of patients versus control groups, All data were presented as mean± SD, HB:Hemoglobin, WBCs: white blood cells, PLT:Platelet, LYM%: lymphocytes relative value . WBCs, PLT were not significant between groups of patients and control subjects P>0.05 and HB, LYM were significant between groups of patients and control subjects(P<0.05)
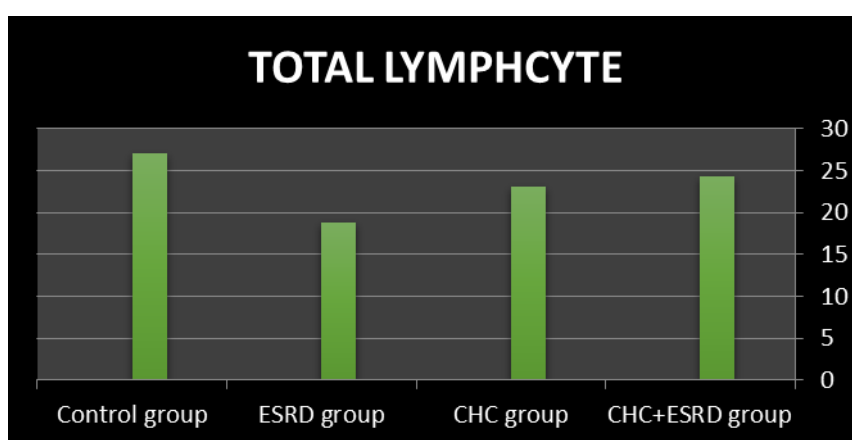

Fig.1: Level of total lymphocytes in different groups of patients and control subjects. Results are expressed as mean ±SD.

Flow Cytometry detection of B-lymphocytes and NK-cells shows that, The percentage of B lymphocytes (CD19^{+}) was significantly increased in (CHC+ESRD) group compared with ESRD group, Normal group (P<0.05). Also, it showed significantly decrease when compared with (CHC) group. NK cells (CD56^{+}) not significantly decreased in (CHC) group compared with (CHC+ESRD) group and control group. Also, it showed not significant decrease also in (CHC+ESRD) group compared with ESRD and control group (P >0.05), The B1 lymphocytes (CD5^{+}) was decreased in (CHC) group compared with Control subjects and decreased in (CHC+ESRD & ESRD) groups compared with control group. No statistically significant difference was shown between groups (P>0.05).

Table 5:- comparison between percentage of B lymphocytes and NK cells in different groups of patients and control persons.

Parameter	CHC+ESRD group	CHC group	ESRD group	Control group	P value
CD5	70.02 ± 8.62	66.93 ± 7.61	70.91 ± 8.60	72.56 ± 4.42	N.S
CD19	5.58 ± 3.43	6.40 ± 1.41	4.29 ± 2.23	4.84 ± 2.48	P<0.05
CD56	16.11 ± 4.82	15.10 ± 2.23	18.15 ± 5.58	18.29 ± 9.35	N.S

All data were presented as mean± SD. CD5 ,CD56 were not significant between groups of patients and control subjects and CD19 was significant between groups of patients and control subjects(P<0.05)
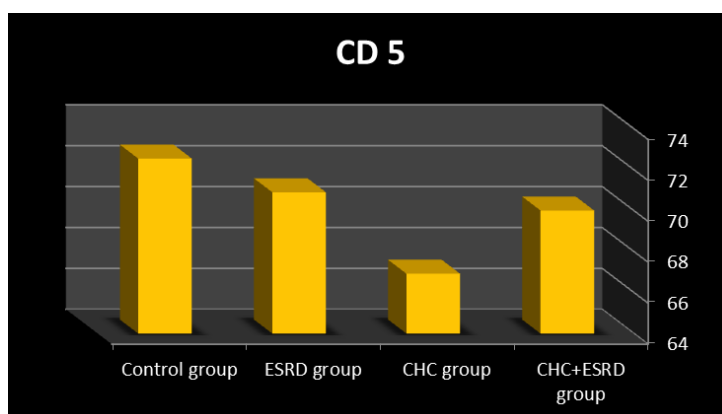
**Fig.2: Level of CD5 in different groups of patients and control subjects.
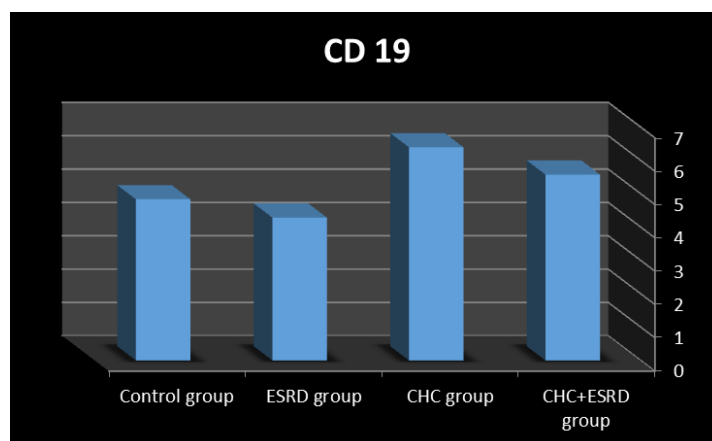Results are expressed as mean ±SD**

**Fig. 3: Level of CD19 in different groups of patients and control subjects.
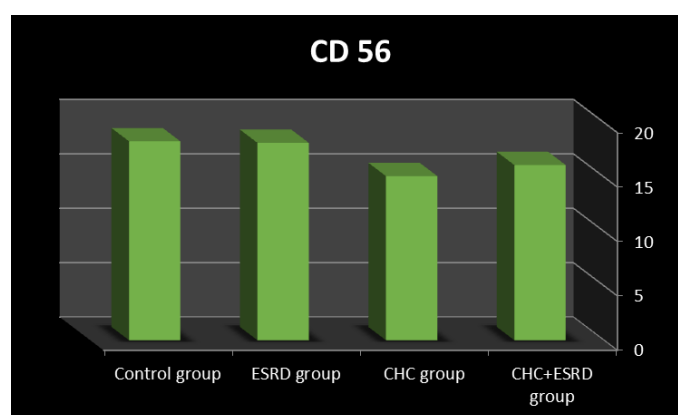Results are expressed as mean ±SD**

**Fig. 4: Level of CD56 in different groups of patients and control subjects.
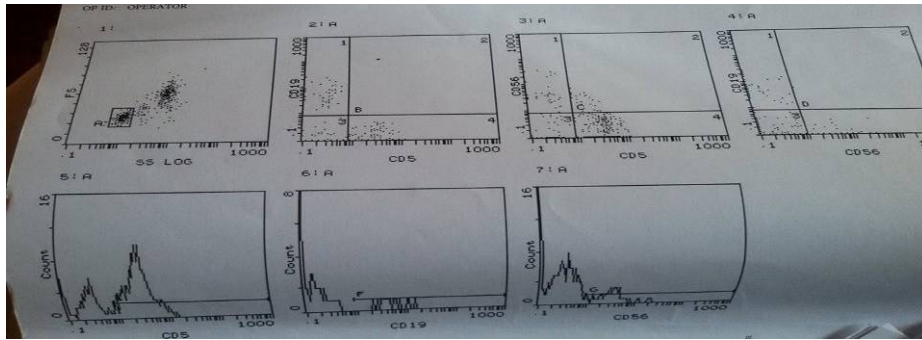Results are expressed as mean ±SD**

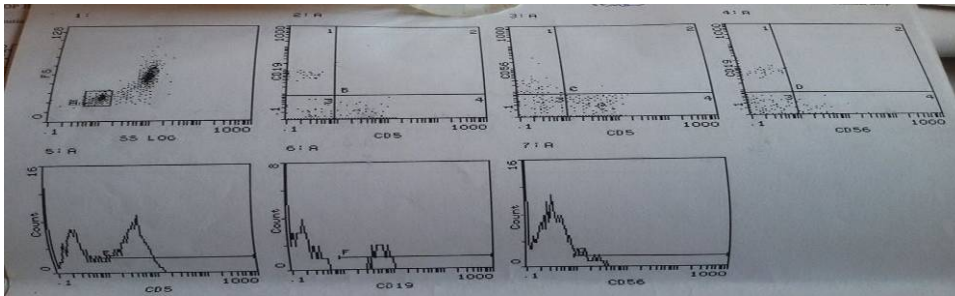**Fig. 5: Scatter gram showing CD5, CD19, CD56 in (CHC+ESRD) group.**

Fig. 6: Scatter gram showing CD5, CD19, CD56 in (CHC) group.
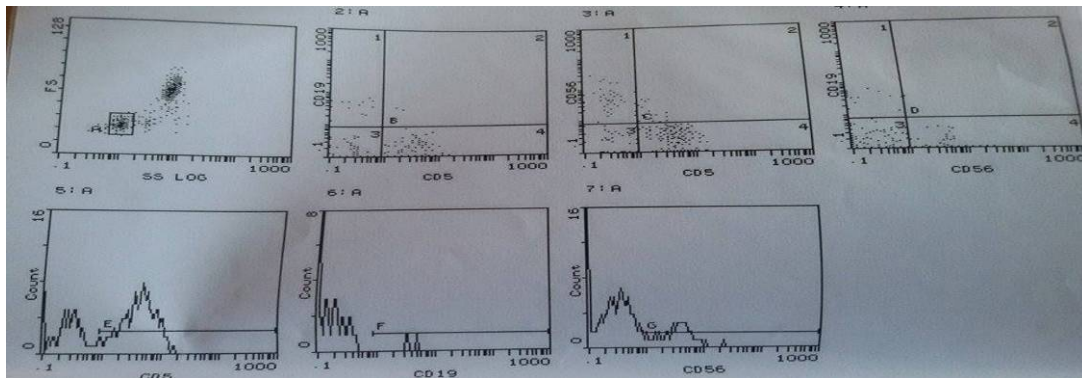

Fig. 7: Scatter gram showing CD5, CD19, CD56 in (ESRD) control group.
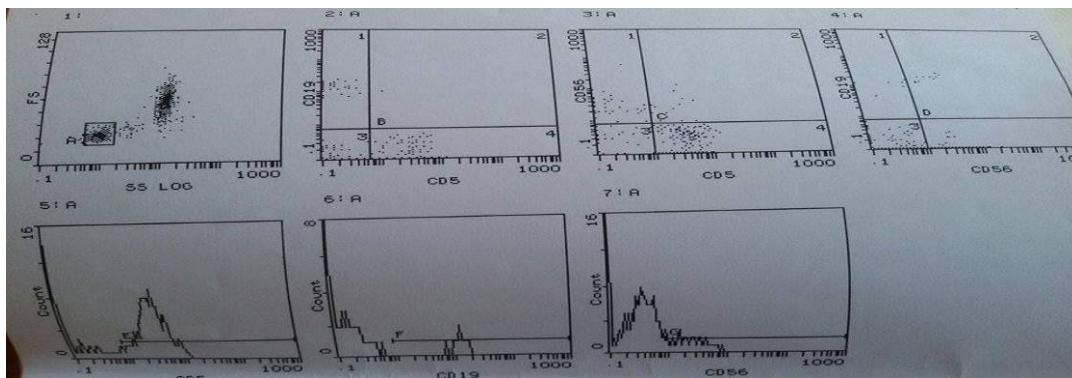

Fig. 8: Scatter gram showing CD5, CD19, CD56 in control group.

DISCUSSION

The aim of this investigation was to demonstrate that CHC and CHC+ESRD patients present a different pattern of immune response as a result of complex mechanisms involving innate (NK"CD56", B1"CD5" Cells) and humoral adaptive immunity (B cells "CD19"). The importance of this lies in the fact that this peculiar immune pattern may be associated with the induction and/or regulation of the pathogenesis of HCV infection in ESRD patients [22,23].

In this study, Most patients have normal ALT levels, treatment naïve uncomplicated chronic hepatitis C patients in 2 groups (CHC, CHC+ESRD), lower HCV-RNA levels as in [14,24].

AST (P<0.01) and ALT (P<0.001) showed statistically increased by HCV infection in (CHC+ESRD group &CHC group) compared with ESRD control group and normal control group. Similar to Sayarlioglu et al.[25].

Serum Urea and creatinine level were higher than normal range in ESRD patients undergoing dialysis. There were significantly increased by ESRD (CHC+ESRD & ESRD group) compared with (CHC group & control group), Urea (P<0.001) and creatinine (P<0.001).similar to Amin et al., [26].

Hemoglobin (Hb) level was found low in ESRD patients due to removal of blood during dialysis. In the current study had a significantly decreased in mean of hemoglobin concentration in (CHC+ESRD & ESRD) groups (P<0.01) compared to control subjects. This low Hb level most of the time led to the development of anemia. Similar to Amin et al"[26].

In this study, Total Lymphocyte count was decreased in (CHC, ESRD & CHC+ESRD) groups compared with control group. Also it, increase in (CHC+ESRD) group compared with CHC group and ESRD. Total lymphocytes were significant (P<0.05) between groups of patients compared with control subjects. As shown in table 4

In contrast to Sayarlioglu et al.[25]. Who stated that, no statistically significant difference has been detected between the lymphocyte counts in HCV positive and negative HD patients compared to healthy control group, lymphocyte counts in HCV positive was detected to be low compared to HCV negative HD patients.

In vivo, intrahepatic NK cell numbers and peripheral blood NK cells expressing perforin are decreased suggesting that cytotoxic function may be altered in patients with chronic HCV infection compared with healthy controls[27]. Chronic HCV infection is associated with reduced NK cell frequency and function (perforin and interferon-γ secretion) in the peripheral blood and in the liver [28]. This study showed, NK cells (CD56^{+}) not significantly decreased in (CHC) group compared with control group. Similar to Wan et al.[29].

In contrast to, Barbosa et al, who stated that, higher frequency of CD56 NK- cells was observed in (CHC+ESRD) as compared to Normal, CHC and ESRD groups. We did not found any significant decrease in NK cells (CD56^{+}), between (CHC+ESRD) group and ESRD group or normal control (P >0.05) similar to what reported by Shiina et al [30]as well as previous reports[31,32,33].

Innate B1 cells (CD5+ B cells) count for 25–27% of peripheral blood B lymphocytes. Innate B1 cells produce mainly IgM antibodies that have high cross-reactivity but low-affinity[34]. These antibodies constitute a readily-available pool of immunoglobulin for use against a variety of infections before the specific high-affinity antibodies are produced. In this study we found statistically non-significant decrease of B1 lymphocytes (CD5+) in (CHC) group compared with control subjects while (CD19+) was significantly increased in (CHC) group compared with control group, which is the same finding of Jianhua et al.[35] who showed that the relative frequency of circulating CD5 B cells was somewhat reduced in HCV patients compared with normal volunteers, although those patients with elevated CD19 B cell frequencies had higher numbers of CD5 B cells as well.

ESRD is simultaneously associated with immune activation which is marked by systemic inflammation and immune deficiency [36,37.] ESRD, and especially HD, is associated with B-cell lymphopenia. It has been suggested that one of the major causes of this disturbance is an increased susceptibility to the death of B cells by apoptosis [38.]

Diminished population of CD5+ innate B cells and CD19 B cells has been demonstrated in chronic renal failure [39]. In a recent study, Pahl et al.[40] demonstrated depletion of several other B cell subtypes in adult patients with ESRD. In this study, CD5 and CD19 were decreased in ESRD group when compared with normal group.

Two alternative mechanisms can account for B lymphopenia in ESRD. First, the uremic milieu may increase susceptibility of B cells to apoptosis in ESRD patients. This supposition is supported by the study of Fernández-Fresnedo et al who reported increased apoptosis of B cells in their CKD patients [38.] The second possibility is that the uremic environment may interfere with the maturation of transitional B cells to mature B cells by promoting resistance to B Cell Activating Factor of tumor necrosis family (BAAF) mediated differentiation and survival signals. This supposition was supported by marked down-regulation of BAAF receptor in ESRD patients reported by Pahl et al [40]. Thus B cell deficiency and dysfunction in advanced CKD can be simultaneously mediated by increased B cell apoptosis and impaired Transitional B cell differentiation and maturation.

As a conclusion, in this study, CD5 showed, not statistically significant decrease in (CHC+ESRD) and (ESRD) groups compared with control group. (CHC+ESRD) group and (ESRD) group were as the similar in CD5 percentage. Suggesting that that Hepatitis C infection poorly affect CD5 value. While CD19 showed significant increase in (CHC+ESRD) group when compared with ESRD group and significant decrease when compared with CHC group. In contrast to Barbosa et al [33], who stated that , The ESRD control group and CHC+ESRD patients presented significant lower frequency of CD19+ B cells as compared to control and CHC groups.

Some studies have shown that the cellular immune response is relatively preserved in ESRD, with concomitant deficiency of the humoral immune response [14,41] Recent study concluded that the viral persistence in dialysis patients is due to a failure of the adaptive immune system, as shown by the absence of significant T-cell and antibody responses, as well as viral variability [42].

We understand that the small number of our samples in this study limited the clarifying of an immunological immune response pattern in CHC and CHC+ESRD patients however we recommend further investigation on larger scale to demonstrate the immune response pattern in such patients.

REFERENCES

1. Cooke GS, Lemoine M, Thursz M, Gore C, Swan T, Kamarulzaman A, et al. Viral hepatitis and the Global Burden of Disease: a need to regroup. J Viral Hepat. 2013;20:600–601.
2. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med. 2001;345:41–52.
3. Moyer VA; U. S. Preventive Services Task Force. Screening for hepatitis C virus infection in adults: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2013;159:349–357.
4. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013;57:1333–1342.
5. Zoheiry, M.M., et al., serum markers of epithelial mesenchymal transision as predictors of HCV-induced liver fibrosis, cirrhosis and hepatocellular carcinoma. 2015.
6. Razavi H, ElKhoury AC, Elbasha E, Estes C, Pasini K, Poynard T, et al. Chronic hepatitis C virus (HCV) disease burden and cost in the United States. Hepatology. 2013;57:2164–2170.
7. Su Y, Norris JL, Zang C, Peng Z, Wang N. Incidence of hepatitis C virus infection in patients on hemodialysis: a systematic review and meta-analysis. Hemodial Int. 2013;17:532–541 Chapter 1: Definition and classification of CKD. Kidney Int Suppl (2011) 2013;3:19–62
8. K. W. Kim, B. H. Chung, E. J. Jeon et al., "B cell-associated immune profiles in patients with end-stage renal disease (ESRD)," Experimental & Molecular Medicine, vol. 44, no. 8, pp. 465–472, 2012.
9. N. D. Vaziri, M. V. Pahl, A. Crum, and K. Norris, "Effect of uremia on structure and function of immune system," Journal of Renal Nutrition, vol. 22, no. 1, pp. 149–156, 2012.
10. S. Kato, M. Chmielewski, H. Honda et al., "Aspects of immune dysfunction in end-stage renal disease, "Clinical Journal of the American Society of Nephrology, vol. 3, no. 5, pp. 1526–1533, 2008.
11. Eleftheriadis T, Antoniadi G, Liakopoulos V, Kartsios C, Stefanidis I: Disturbances of acquired immunity in hemodialysis patients. Semin Dial20 :440– 451,2007
12. Fernandez-Fresnedo G, Ramos MA, Gonzalez-Pardo MC, de Francisco AL, Lopez-Hoyos M, Arias M: B lymphopenia in uremia is related to an accelerated in vitro apoptosis and dysregulation of Bcl-2. Nephrol Dial Transplant15 :502– 510,2000
13. Walker, C.M., Adaptive immunity to the hepatitis C virus. Adv Virus Res, 2010. 78: p. 43-86
14. Meyers, C.M., et al., Hepatitis C and renal disease: an update. American journal of kidney diseases, 2003. 42(4): p. 631-657.
15. Poordad, F.F., F. Fabrizi, and P. Martin. Hepatitis C infection associated with renal disease and chronic renal failure. in Seminars in liver disease. 2004; 24: 69–77.
16. Freeman, A.J., et al., Immunopathogenesis of hepatitis C virus infection. Immunology and cell biology, 2001. 79(6): p. 515-536.
17. Darling, J.M. and T.L. Wright, Immune responses in hepatitis C: is virus or host the problem? Current opinion in infectious diseases, 2004. 17(3): p. 193-198.
18. Berthelot, J.-M., et al., Regulatory B cells play a key role in immune system balance. Joint Bone Spine, 2013. 80(1): p. 18-22.
19. Ronet, C., et al., Regulatory B cells shape the development of Th2 immune responses in BALB/c mice infected with Leishmania major through IL-10 production. The journal of immunology, 2010. 184(2): p. 886-894.
20. Apolinario, A., et al., Increased expression of T cell chemokines and their receptors in chronic hepatitis C: relationship with the histological activity of liver disease. The American journal of gastroenterology, 2002. 97(11): p. 2861-2870.
21. Calvino, M., et al., role of CCR5 and CXCR3 chemkine receptors expression on CD8+ cells

during chrnic hepatitis infection. Journal of Hepatology, 2007. 46: p. S170

22. Herkel J., et al., Immune-mediated liver injury. Journal of hepatology, 2005. 42(6): p. 920- 923.

23. Doherty, D.G. and C. O'Farrelly, Innate and adaptive lymphoid cells in the human liver. Immunological reviews, 2000. 174(1): p. 5-20

24. Okuda K, Yokosuka O. Natural history of chronic hepatitis C in patients on hemodialysis: case control study with 4–23 years of follow-up. World J Gastroenterol 2004; 10: 2209–12

25. Hayriye Sayarlioglu,Reha Erkoc,Ekrem Dogan,Yasemin Syral,Ahmet Faik Oner, lymphocyte subtybe and immungloobulins levels in HCV possitive hemodialysis pateints,Eur j Gen med ,2012;9(3):183-186

26. Noor ul Amin, Raja Tahir Mahmood*, M. Javaid Asad, Mudassar Zafar, and Asad Mehmood Raja, Evaluating Urea and Creatinine Levels in Chronic Renal Failure Pre and Post Dialysis: A Prospective Study, JOURNAL OF CARDIOVASCULAR DISEASE ,2014; 2330-459

27. Deignan T, Curry MP, Doherty DG, et al., Decrease in hepatic CD56(+) T cells and V alpha 24(+) natural killer T cells in chronic hepatitis C viral infection. J Hepatol.(2002);37(1):1018.

28. Meier UC, Owen RE, Taylor E, et al., Shared alterations in NK cell frequency, phenotype, and function in chronic human immunodeficiency. virus and hepatitis C virus infections. J Virol,(2005); 79:12365–74

29. Wan H, Liy, Yang N, Yuan W, Xie N, Wang D, Guo T, Mao Y, Changes of lymphocyte subsets in peripheral blood of patients with hepatitis C, 2014;30(10):1058-61

30. Masaaki Shiina, Koju Kobayashi, Kazumasa Hirishi, and Michio Imawari. hepatitis C patients on maintenance hemdialysis show cmplex immune disturbances in the peripheral blood.viral immunology 2013 0026

31. Golden –Mason L,Madrigal-Estebas L,McGrath E,et al. Al-tered natural killer cell subset2 distribution in resolved and persistent hepatitis C virus infection following single source exposure .Gut 2008;57:1121-1128.

32. Hinrichsen H,Leimenstoll G,Stegen G,et al.,clinical presentation of chronic hepatitis C in patients with end-stage renal disease and on hemodialysis versus those with normal renal function. Am J Gastroenterol 2005;100:2010-2018.

33. katia V.B.D. Barboosa, Rosangela Teixeira, Eric Bassetti-Soares, Aecioo. Meirelles de Souza ,Joao Milton M.O. Penido, Andrea Teixira-carvalho and Olindo A. Martins-filho, phenotypic features of innate and adaptive immunity in patients with chronic hepatitis C and end stage renal disease.Liver international.2013;33:1349-1356

34. Herzenberg L, Haughton G, Rajewsky K. CD5 B cells in development and disease. Ann NY Acad Sci.1992;651:591–601

35. Jianhua Ni, Edgardo Hembrador, Adrian M. Di Bisceglie, Ira M. JacobsonAndrew H. Talal, David Butera, Charles M. Rice, Thomas J. Chambers, and Lynn B. Dustin. Accumulation of B Lymphocytes with a Naive, Resting Phenotype in a Subset of Hepatitis C Patients. J Immunol 2003; 170:3429-3439

36. Carrero JJ, Stenvinkel P. Inflammation in end-stage renal disease--what have we learned in 10 years? Semin Dial. 2010 Sep–Oct;23(5):498–509

37. Girndt M, Sester U, Sester M, Kaul H, Kohler H. Impaired cellular immunity in patients with end-stage renal failure. Nephrol Dial Transplant. 1999;14:2807–2810

38. Fernandez-Fresnedo G, Ramos MA, Gonzalez-Pardo MC, de Francisco AL, Lopez-Hoyos M, Arias M: B lymphopenia in uremia is related to an accelerated in vitro apoptosis and dysregulation of Bcl-2. Nephrol Dial Transplant15 :502– 510,2000

39. Bouts A, Davin J, Krediet R, et al. Children with chronic renal failure have reduced numbers of memory B cells. Clin Exp Immunol. 2004;137:589–594

40. Pahl MV, Gollapudi S, Sepassi L, Gollapudi P, Elahimehr R, Vaziri ND. Effect of end-stage renal disease on B-lymphocyte subpopulations, IL-7, BAFF and BAFF receptor expression. Nephrol Dial Transplant. 2010 Jan;25(1):205–12

41. Hinrichsen H, Leimenstoll G, Stegen G, Schrader H, F€olsch UR, Schmidt WE; PHV Study Group. Prevalence and risk factors of hepatitis C virus infection in haemodialysis patients: a multicentre study in 2796 patients. Gut 2002; 51: 429–33.

42. Lukas W, Neumann-Haefelin C, Viazov S, et al. Acute infection with a single hepatitis C virus strain in dialysis patients: analysis of adaptive immune response and viral variability. J Hepatol 2009; 50: 693–704.