ORIGINAL ARTICLE

LEVELS OF INTERLEUKIN-7 IN UNTREATED AND INTERFERON TREATED PATIENTS OF HEPATITIS-C: A CROSS-SECTIONAL STUDY

Alamzeb Jadoon, Ammar bin Saad*, Muhammad Basharat Khan*, Muhammad Zahid, Jamil Ahmed Kiyani, Nisar Ahmed*****

Department of Physiology, Nowshera Medical College, Nowshera, *Department of Pathology, Ayub Medical College, Abbottabad, Department of Physiology, AJK Medical College, Muzaffarabad, ***Department of Pharmacology, Ayub Medical College, Abbottabad, Pakistan

Background: Hepatitis C is chronic viral disease that affects liver cells. Interleukin-7 (IL-7) is an important marker in adaptive immunity as it helps immunity to fight various infections as it acts as a growth and survival factor for T lymphocytes. It improves adaptive immunity towards viral infections like hepatitis C itself. In Hepatitis C patient, its role remains debatable. In our research, we studied the levels of serum IL-7 in Control group, interferon (IFN) treated and untreated groups of Hepatitis C in order to assess the effect of IFN treatment on the indicators used. Objective of this study was to measure and compare the levels of IL-7 in treated and untreated patients of Hepatitis C. **Methods:** After written, informed consent, 26 subjects in each group (Control, Untreated HC patients and Interferon Treated HC patients) of both sexes were recruited from all districts of Hazara Division. The PCR was done for viral load. Serum IL-7 level was measured using ELISA. Data obtained were analysed using SPSS-20 and was compared within three groups. **Results:** Mean serum levels of IL-7 measured in in treated group were higher as compared to untreated groups. No statistically significant difference was observed in the serum levels of IL-7 between any of the three groups studied. **Conclusions:** The study showed no significant differences on comparison in IL-7 levels within control, untreated and interferon treated groups. However, with a higher number of samples may define he role of IL-7 in HC.

Keywords: Hepatitis C; Interferon; Interleukin-7; patients; Comparison

Pak J Physiol 2022;18(1):26–8

INTRODUCTION

Hepatitis C (HC) is a chronic disorder of health that affects approximately 184 million worldwide.[1] Pakistani population has a prevalence rate of about 5%.[2] Hepatitis C virus (HVC) has got four main genotypes that are 1–4 and more than 50 sub genotypes. Out of these genotypes, 3a is most common in Pakistan.[3]

INF is a cytokine that is normally produced during any infection inside the body by white blood cells. INF along with antiviral agent Ribavirin was the main stay in the treatment of HC but has been replaced by sofosbuvir recently.[4] HCV infection is diagnosed by using Enzyme linked immunoassay (ELISA) followed by quantitative and qualitative PCR. Quantitative PCR gives an estimated viral load or number of viruses in the blood of the affected person. IFN treated patient should ideally have undetected viral load at the end of treatment. ETR is the amount of viral load at the end of treatment and SVR is the viral load 24 weeks after treatment. ETR and SVR are the markers for the success of IFN treatment in any HC patient.[5] Nearly 80% of the HC infections end up in to chronic stage and only 20 per cent infections are spontaneously resolved with or without treatment in the acute stage.[6]

IL-7 is a cytokine that helps in the proliferation and survival of T lymphocytes in the blood thus may contribute to the eradication of viral infection by cell mediated immunity. IL-7 is the factor that may improve or help clearance of virus by promoting helper and cytotoxic T lymphocytes survival, function and maintaining their number.[7,8] Cytotoxic T lymphocytes are the main antiviral immune cells in human body. These are effective and higher in number at early stage of the disease however they lose their potential function and decrease in number as the disease progress. IL-7 plays a vital role in adoptive immunity and augments immunity to viral infections and tumors.[7]

IL-7 immunotherapy will be of substantial benefit in the treatment of HIV/HCV co-infection and should enhance the likelihood of HCV eradication in poorly responding patients.[9] HCV had got better response to interferon treatment that may be due to higher IL-7 levels.[10] This rationale led the current study and aimed to find and compare the level of IL-7 in treated and untreated patients of the HCV was measured and compared.

SUBJECTS AND METHODS

This cross-sectional study, based on a total of 80 adult subjects from Hazara division of Khyber Pakhtunkhwa province, was approved by the Ethical Committee and Research Board of the University of Health Science, Lahore. During 2017–18, Consecutive random sampling was used for recruiting the patients into the

study and informed written consent (Urdu language) was explained and signed by all subjects.

The study subjects were divided into the following three groups. Group I: Controls with normal LFT's and negative PCR for HCV (n=28). Group II: Untreated patients of HC that had no prior INF treatment (n=26). Group III: INF treated cases of HC who have recently completed their treatment (n=26)

Inclusion criteria was the untreated cases, diagnosed cases of HCV based on ELISA/PCR, adults of both sexes of 18–60 years, and fresh cases of HCV were included. Similarly, inclusion criteria for treated cases were diagnosed cases of HCV based on ELISA/PCR, adults of both sexes of 18–60 years of age and treated cases of HCV who completed interferon treatment in the last week. Patients who were obese, had BMI>25, diabetics, had Hepatitis A and/or B positive, and any other disease known to interfere with HCV were excluded from the study.

Sampling was done at Hepatitis Centre at DHQ Hospital, Abbottabad and the samples were carried to University of Health Sciences, Lahore with ice packs maintaining a cold chain. After 10–12 hours of overnight fast, 5 ml of blood was drawn from a superficial vein with aseptic techniques. Fasting blood glucose was checked with a glucometer (Xceed, Abbott®). Blood was secured in serum separation tubes (SST) vacutainers (yellow top) for extraction of serum. Serum was extracted by centrifuging the blood for 10 minutes at 3,000 rpm. The serum was put in aliquotes and stored at -80 °C till analysis. All other tests were conducted on serum. Serum IL-7 levels were measured using commercially available ELISA kits (Glory Science Co., USA®).

The data were analyzed on SPSS-20. Mean±SD was given for quantitative variables (age, IL-7 levels in serum). Frequencies and percentages were given for qualitative variables (gender, sample group). The data was given either as Mean±SD for normally distributed variables, or median interquartile range (IQR) for non-normally distributed variables. The comparisons of various variables for association or significance were done to see any significant difference in their means/medians. In case of normally distributed data, single factor ANOVA was applied followed by Tukey's post hoc test for the three groups. Independent *t*-test was used for comparing means of two groups, and $p \leq 0.05$ was considered statistically significant.

RESULTS

Out of total 80 samples, 31 (38.8%) were male and remaining 49 (61.3%) were female. Median (IQR) age of male in control group was 31.50 (24.25–34.23) years, and 28.50 (20.00–38.75) years for female.

Level of IL-7 levels of control, untreated and treated group were 44.63±22.11, 35.11±14.68 and 37.87±17.66 respectively, whereas Median (IQR) was 39.95 (31.62–53.49) for controls, 32.80 (22.86–46.19) for untreated and 40.54 (20.05–51.91) showing no significant difference (Kruskal-Wallis test; $p = 0.214$) (Table-1).

IL-7 level of males in control group was 40.63±18.95 pg/mL and female 47.12±24.11 pg/mL showing no significant difference (Independent sample *t*-test; $p=0.478$). Serum IL-7 levels of males in untreated group were 32.50±17.03 pg/mL and for female it was 36.98±13.09 pg/mL showing no significant differences (Independent sample *t*-test; $p=0.437$ (Table-2).

There were no significant differences between levels of IL-7 of controls and treated/ untreated patients (Mann-Whitney U Test; $p=0.624$, and $p=0.113$ respectively). Also, no significant differences of IL-7 levels were found between treated and untreated cases (Independent sample *t*-test; $p=0.528$). Post hoc comparison of age for control and treated/untreated cases showed significant differences (Mann-Whitney U Test; $p<0.001$). There was non-significant negative correlation found between age and IL-7 in control group *(*Spearman Rho correlation; -0.226; Mann-Whitney U Test; $p=0.267$).

Table-1: Comparison of IL-7 between controls, treated and untreated groups by Kruskal-Wallis test (pg/mL)

Parameter	Controls (N=26)	Untreated Cases (N=26)	Treated Cases (N=28)	*p*
Mean±SD	44.63±22.11	35.11±14.68	37.87±17.66	0.342
Median (IQR)	39.95 (31.62–53.49)	32.80 (22.86–46.19)	40.54 (20.05–51.91)	

Table-2: Comparison of IL-7 among controls, untreated and treated groups [Mean±SD, Mean (IQR)]

Parameters	Gender	N	Controls (N=26)	*p*	N	Untreated Cases (N=26)	*p*	N	Treated Cases (N=28)	*p*
IL-7 (pg/mL)	Male	12	40.63±18.95 38.71 (27.65–53.49)	0.478	12	32.50±17.03 29.70 (17.43–42.89)	0.437	12	42.83±18.81 42.78 (27.17–57.56)	0.266
	Female	14	47.12±24.11 39.95 (32.80–68.88)		14	36.98±13.09 35.47 (25.33–7.45)		16	34.76±16.75 36.78 (20.05–51)	

DISCUSSION

According to best of our knowledge, there is no such study conducted previously anywhere in HCV patients for levels of IL-7 in untreated and treated HCV cases to observe the effect of interferon treatment. In current study, there was notable difference in mean levels of IL-7 in controls untreated cases and treated cases but it was not statistically significant ($p<0.342$).

Lundstrom *et al*[12], reported serum IL-7 levels of 2–8 pg/mL in healthy individuals and as high as 60 pg/mL in lymphopenia. The level of IL-7 in our healthy control group were 44±22.11 pg/mL, while in untreated and treated groups the levels of IL-7 were 35.11±14.68 and 37.87±17.66 pg/mL respectively.

A non-significant correlation of IL-7 with age was found in controls and untreated subjects. This observation points to the fact that there is a decrease in immunity of the body with increasing age as decreasing IL-7 levels fail to maintain the number of T lymphocytes required for the immunity of the body. IL-7 contributes to both innate and acquired immunity by increasing proliferation and inhibiting apoptosis of T lymphocytes. According to Bolotin *et al*, IL-7 levels were lower in adults (2.82 pg/mL) as compared to children (4.8 pg/mL; $p<0.05$).[11]

CONCLUSION

No significant differences on comparison in IL-7 levels within controls, untreated and interferon treated groups and could not provide any mounting evidence as far as the function of IL-7 in Hepatitis C is concerned.

REFERENCES

1. Jafri SM, Gordon SC. Epidemiology of Hepatitis C. Clin Liver Dis (Hoboken) 2018;12(5):140–142.
2. Al Kanaani Z, Mahmud S, Kouyoumjian SP, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Pakistan: systematic review and meta-analyses. R Soc Open Sci 2018;5(4):180257.
3. Umer M, Iqbal M. Hepatitis C virus prevalence and genotype distribution in Pakistan: Comprehensive review of recent data. World J Gastroenterol 2016;22(4):1684–700.
4. Hézode C. Treatment of hepatitis C: Results in real life. Liver Int 2018;38(Suppl 1):21–7.
5. Guss D, Sherigar J, Rosen P, Mohanty SR. Diagnosis and Management of Hepatitis C Infection in Primary Care Settings. J Gen Intern Med 2018;33(4):551–7.
6. Gardenier D and Olson MC. Hepatitis C in 2019: Are We There Yet? J Nurs Pract 2019;15(6):415–9.
7. Hotchkiss RS, Steven M. Opal SM. Activating immunity to fight a foe – A New Path. N Engl J Med 2020;382:1270–2.
8. Zhang ZH, Jiang BC, Liu XH, Zhang MX, Li ZSN, Zhu GZ. Interleukin-7 Regulates T Follicular Helper Cell Function in Patients with Chronic Hepatitis C. Viral Immunol 2018;31(6):417–25.
9. Francois B, Jeannet R, Daix T, Walton AH, Shotwell MS, Unsinger J, *et al*. Interleukin-7 restores lymphocytes in septic shock: the IRIS-7 randomized clinical trial. JCI Insight 2018;3(5):e98960.
10. Chaturvedi N, Svarovskaia ES, Mo H, Osinusi AO, Brainard DM, Subramanian GM, McHutchison JG, Zeuzem S, Fellay J. Adaptation of hepatitis C virus to interferon lambda polymorphism across multiple viral genotypes. Elife 2019 Sep 3;8:e42542.
11. Bolotin E, Annett G, Parkman R, Weinberg K. Serum levels of IL-7 in bone marrow transplant recipients: relationship to clinical characteristics and lymphocyte count. Bone Marrow Transplant 1999;23(8):783–8.
12. Lundström W, Fewkes NM, Mackall CL. IL-7 in human health and disease. Semin Immunol 2012;24(3):218–24.

Address for Correspondences:

Dr. Muhammad Basharat Khan, Assistant Professor, Chemical Pathology, Department of Pathology, Ayub Medical College, Abbottabad, Pakistan. **Cell:** +92-345-9000501
Email: mohdbasharat81@gmail.com

Received: 7 Nov 2021 **Reviewed:** 12 Jan 2021 **Accepted:** 18 Jan 2022

Contribution of Authors:
AJ: Design, Concept, data acquisition, and script writing
ABS: Drafting and critically revising
MBK: Data acquisition, analysis and result
MZ: Study design and discussion writing
NA: Proof reading, result analysis and final approval
JAK: Literature search and review

Conflict of Interest: None to declare
Funding: None