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Immune Response Of Hepatitis C Virus Infected Patients, Jazan Region, Saudi Arabia

Yehya Hejri[1]*, Ishtiag M. Qadri[1], Hussein A. Al-Mehdar[1], Naser A. Al-Kenan[2], Abdulaziz M. Hakami[2], Abdulrahman J. Harbi[1], Turki A. Mudabbesh[1], Tareq K. Khawaji[1]

[1]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia.
[2] Jazan Health Directorate, Ministry of Health, Jazan, Saudi Arabia.
*Email: yehyahejri@gmail.com

Abstract:

CD4 T cells are essential for coordinating the immune response and providing help to other immune cells, while CD8 T cells are responsible for directly eliminating virus-infected cells. The interplay between CD4 and CD8 T cells is crucial for an effective immune response. A hospital-based prospective study on 40 hepatitis HCV patients in Jazan region to assess the host immune response was conducted and analyzed. The results indicate a significant decrease in both CD4 and CD8 cell counts following HCV infection. The moderate positive correlation observed between CD4 counts pre and post-infection suggests that higher CD4 counts prior to infection may contribute to relatively higher CD4 counts during the post-infection stage. However, no significant correlation was found between CD8 counts pre and post-infection. These findings highlight the impact of HCV infection on the patient's immune response and the potential importance of CD4 T cells in the immune response against HCV. The current studies examining the counts of CD4 T and CD8 T cells in 40 patients have indicated the significance of these HCV-specific T cells in the immune response against HCV. Although, the findings obtained suggest that the timing and coordination of the expansion of these T cell responses during primary and chronic HCV infection could influence the effectiveness of controlling the initial infection. Further research and analysis are needed to fully understand the impact of HCV infection on T cell populations and their functional characteristics.

Key words: Hepatitis C Virus, Immune response, CD4 and CD8, Jazan region, Saudi Arabia.

INTRODUCTION:

Hepatitis C Virus (HCV) is an enveloped single stranded positive sense-RNA virus that primarily infects liver cells (Lapa et al.,2019) The virus displays substantial genetic variabilities, contributing to its ability to invade the host immune responses resulting in chronic infection and causes chronic liver disease (Ma et al.,2006). The infected host immune response plays a critical role in controlling and clearing HCV infection (Ryscavage et al., 2024). The first line of defense against HCV infection is the innate immune response. Many cells including dendritic cells, natural killer (NK) cells, and macrophages recognize viral components through pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and RIG-I-like receptors (RLRs) (Joyce et al.,2019). These immune cells produce pro-inflammatory cytokines and chemokines, promoting an antiviral state and recruiting other immune cells to the site of infection (Joyce et al., 2019). The infected host adaptive immune response is characterized by the activation of antigen-specific immune cells including B cells and T cells (Joyce et al., 2019). Therefore, adaptive immune response is crucial for the clearance of HCV infection. The immunoglobulins antibodies produce by B cells produce antibodies neutralize HCV and prevent its spread. Because, the HCV has the ability to evade neutralizing antibodies through high genetic variability and glycan shielding, making the development of broadly neutralizing antibodies challenging (Lapa et al., 2019). The crucial role of Helper T cells (CD4+) is the adapting and orchestrating the immune response against HCV involves their recognition of viral antigens displayed on antigen-presenting cells (APCs) through their T cell receptors (TCRs). CD4+ T cells not only aid B cells in producing antibodies but also facilitate the activation of CD8+ T cells. The CD8+ T cells, referred to as cytotoxic T lymphocytes (CTLs), play a vital role in eradicating HCV-infected cells (Guérin et al., 2024). They identify viral antigens displayed on the surface of infected cells and release cytotoxic molecules, such as perforin and granzymes, to initiate cell death. Due to the significant genetic variability of HCV, multiple viral variants are generated that can evade detection by CD8+ T cells, allowing the virus to persist (Obeagu and Obeagu, 2024). Cytokines is small proteins, play a vital role in regulating and coordinating immune responses (Mosa et al., 2023). In the context of HCV infection, various cytokines such as interferons (IFNs), interleukins (ILs), and tumor necrosis factor-alpha (TNF-α) are generated, stimulating antiviral activity, inflammation, and the activation of immune cells (Park and Hahn, 2023). Tregs is specific subset of CD4+ T cells, have the function of suppressing immune responses and contributing to the maintenance of immune balance. During HCV infection, they play a crucial role in regulating the immune response to prevent excessive inflammation and immunopathology (Nie et al., 2023). Although liver is the primary location of HCV infection, possesses distinct immune features. Therefore, it harbors specialized immune cells, including liver-resident macrophages (Kupffer cells) and liver-specific NK cells, which play a role in the immune response against HCV within the liver microenvironment. In addition the host immune response plays a crucial role in controlling HCV infection, the virus has developed multiple strategies to evade or manipulate the immune response, resulting in chronic infection in a significant number of individuals (Park and Hahn, 2023). There is a lack of data concerning the immune-response of HCV **infected patients** in the Jazan region. Therefore, the current patient immune response patterns and tailoring appropriate interventions for HCV-infected individuals in a particular region were highly needed. Such investigations are crucial and highly necessary to inform the development of effective prevention and control strategies, ultimately reducing the burden of HCV infection in the region.

Objectives

To assess the level of protective immunity against HCV in clinically infected patients admitted to the Jazan General Hospital.

To identify the socioeconomic factors impact the immune response and disease progression among HCV-infected patients in the Jazan region.

MATERIALS AND METHOD:

Study Design

To achieve the research objectives: The host immune response and their socioeconomic factors impact analysis of the hepatitis HCV in Jazan area, KSA, a hospital-based prospective study was conducted.

Study area and population

Jazan region is encompassed a wide range of residential areas including urban, suburban, and rural regions, thereby providing a diverse population for the research. The study participants consisted of individuals seeking medical care in both inpatient and outpatient hospital departments, encompassing people of all nationalities, genders, and adults (above 18 years of age). To ensure a representative sample, participants were selected using a random sampling technique, aiming to include individuals from urban, suburban, and rural areas.

Sample

A total of 52 HCV clinically infected patients - that had previously been positively tested by HCV ELISA - who were admitted to Jazan General Hospital were randomly selected. Approximately 5 ml of blood was collected in tubes containing Ethylenediaminetetraacetic acid (EDTA) and the plasma was stored at -70° C until use.

Sample preparation:

Plasma samples collected from participants were used for HCV detection. Prior to analysis, the -70° C frozen samples were thawed and brought to room temperature. Each sample was assigned a unique identifier to maintain confidentiality.

ELISA kit selection:

A commercially available ELISA kit (DIA.PRO, Italy) specific for the detection of HCV antibodies was selected. The kit was chosen based on its sensitivity, specificity, and compatibility with the study objectives. The kit included all necessary reagents for the assay, including HCV antigen-coated microplates, enzyme-conjugated anti-human IgG antibodies, substrate solutions, and wash buffers.

Plate coating:

The microplates provided in the ELISA kit were coated with HCV antigens. The coating process was performed according to the manufacturer's instructions. Briefly, the microplates were incubated with the HCV antigen solution at an appropriate concentration and temperature for a specified period. After coating, the plates were washed to remove unbound antigen and blocked to prevent nonspecific binding.

Sample incubation:

Plasma samples were added to the coated microplates and incubated under appropriate conditions. The plates were sealed and incubated for a predetermined period to allow the antibodies present in the samples to bind to the immobilized HCV antigens. This step facilitated the specific detection of HCV antibodies in the plasma.

Washing:

After the incubation period, the microplates were washed with a suitable wash buffer to remove any unbound proteins or antibodies. Washing steps were performed multiple times to ensure the removal of nonspecifically bound components.

Enzyme conjugated antibody incubation:

Following the washing step, enzyme-conjugated anti-human IgG antibodies were added to the microplates. These antibodies specifically bind to the HCV antibodies that were captured during the previous incubation step. The plates were incubated again to allow the formation of antibody-enzyme conjugate complexes.

Color development:

After the incubation, a substrate solution containing a chromogenic substrate was added to the microplates. The enzyme attached to the antibody-antigen complex catalyzed a color-producing reaction when in contact with the substrate. The color development was allowed to proceed for a specific duration. To stop the color development reaction, a stop solution was added to the microplates. The stop solution changed the pH, thereby terminating the enzymatic reaction. The optical density of the resulting colored solution was measured using a microplate reader at a specific wavelength recommended by the manufacturer.

Positive and negative ccontrols:

Positive and negative controls provided in the ELISA kit were included in each assay run. The positive control contained known HCV antibodies, while the negative control lacked HCV antibodies. These controls served as references for determining the sensitivity and specificity of the assay and for validating the assay's performance. The OD values obtained from the microplate reader were recorded for each sample. The OD values of the positive and negative controls were also recorded. Data analysis was performed by comparing the OD values of the samples with the cutoff value provided by the manufacturer. Samples with OD values above the cutoff were considered positive for HCV antibodies, while those below the cutoff were considered negative.

Immune response assessment:

HCV specific types of immune cells (CD4 and CD8) involved in the immune response against the infection were tested using BD Multitest™ 6-Color TBNK and BD Trucount™ according to the manufacture procedures. Briefly, plasma samples from infected individuals were centrifuged and cellular debris was removed. Clarified samples were aliquoted into appropriate tubes and store at the recommended temperature until analysis. The microplate was prepared by adding the capture antibody specific for the cytokine of interest to each well. The plate was incubated at 37°C for one hour to allow the capture antibody to bind to the plate surface. The plate was washed with the wash buffer to remove unbound antibodies. HCV test samples and positive controls were added to the wells of the microplate and incubated to allow the cytokines in the samples to bind to the capture antibodies. The plate was washed multiple times with the wash buffer to remove unbound substances. The detection antibody specific for the cytokine of interest was added to each well and incubated to allow the detection antibody to bind to the captured cytokines. The plate was washed again to remove unbound detection antibodies. The substrate solution was added that reacts with the detection antibody to generate a measurable signal (color change) in proportion to the amount of cytokine present and was incubated to allow the reaction to occur. The measure the absorbance or fluorescence of each well was read at the appropriate wavelength and the data obtained from the plate reader was recorded. Data analysis was performed using the provided standards or positive controls to generate a standard curve for cytokine quantification. The cytokine concentrations in the HCV samples were calculated analyzed and interpreted the data using appropriate statistical methods and software.

Ethical approval and informed consent

The study protocol was reviewed and approved by Research Ethic Committee, Jazan Region Ministry of Health, Saudi Arabia.

Informed consent was obtained from all participants before their inclusion in the study, ensuring their voluntary participation and confidentiality of their information.

RESULTS:

The outcome of HCV infection associated to the patient immune-response was assessed by correlation of both CD4 and CD8 T-cells count during the initial and post HCV infection stages.

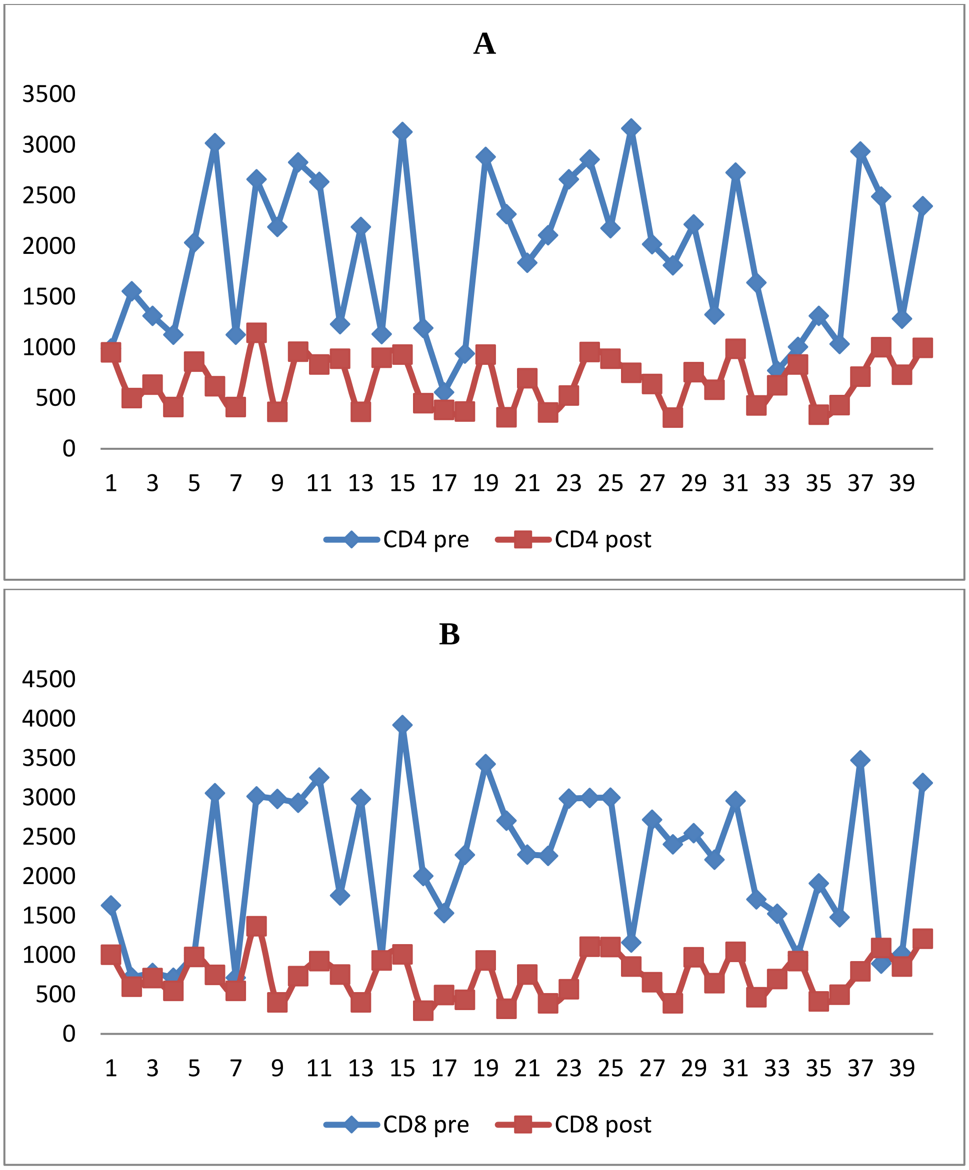
The CD4 and CD8 counts pre-infection and post-infection were sharply decreased (about 300 times) and the CD4/CD8 ratio was also decreased by more than 100% (table 1). Figure 1: The mean CD4 and CD8 counts over time in pre and post HCV infection stages was illustrated in figure 1. The outcome of HCV infection associated to the patient immune-response was assessed by correlation of both CD4 and CD8 T-cells count during the initial and post HCV infection stages.

Table 1: CD4 and CD8 count and their ratio in pre and post HCV infection.

Paired samples	Pre		Post		Decrease	%
	Mean	± SD	Mean	± SD		
CD4	1920.93	758.5	669.17	246.6	551.76	287.06
CD8	2153.19	933.6	739.75	273.3	1413.44	291.07
CD/CD8 Ratio	1.03	647.2	0.91	231.8	0.12	113.19

Table 2: CD4 and CD8 outcome of HCV infection correlations.

	Outcome of HCV infection	N	Correlation	Significant
Pair 1	CD4 pre and CD4 post	40	0.419	0.007
Pair 2	CD8 pre and CD8 post	40	0.170	0.295



DISCUSSION

In HCV infection, both CD4 and CD8 cells play important roles in the immune response against the virus (Lin et al, 2024). CD4 T cells, also known as helper T cells, are crucial for coordinating the immune response against HCV (Bader and Farouk, 2024.). They recognize viral antigens presented by antigen-presenting cells (APCs) and provide help to other immune cells. CD8 T cells, also known as cytotoxic T cells or killer T cells, are responsible for directly killing virus-infected cells. They recognize viral antigens presented on the surface of infected cells in complex with major histocompatibility complex class I (MHC-I) molecules. CD8 T cells are critical for controlling HCV infection (Gridley et al., 2024).

During acute HCV infection, CD8 T cells are activated and expand to eliminate infected hepatocytes (liver cells). However, in chronic HCV infection, the virus can evade the immune response and establish persistent infection. This can lead to functional exhaustion of CD8 T cells, where they become less effective in killing infected cells and producing antiviral cytokines. The interplay between CD4 and CD8 T cells is essential for an effective immune response against HCV (Gridley et al., 2024). CD4 T cells provide help to CD8 T cells by producing cytokines, promoting their expansion, and enhancing their cytotoxic functions. Impaired CD4 T cell responses can affect the overall immune response and contribute to viral persistence (Woo and Choi, 2024). Additionally, CD4 and CD8 cell counts alone do not provide a complete picture of immune function. Other factors, such as the CD4/CD8 ratio and functional characteristics of T cells, also play a role in assessing immune health and response to infections (Gridley et al., 2024, Lin et al, 2024). The presented results indicate the assessment of the patient's immune response to HCV infection by correlating the counts of CD4 and CD8 T cells during the initial and post-infection stages (Woo and Choi, 2024). The data in Table 1 shows a significant decrease in both CD4 and CD8 cell counts post-infection compared to pre-infection levels. Specifically, CD4 counts decreased by an average of 551.76 cells, representing a decrease of approximately 300 times, while CD8 counts decreased by an average of 1,413.44 cells, showing a similar substantial reduction. In addition, the CD4/CD8 ratio decreased by more than 100% (Woo and Choi, 2024). Table 2 presents the correlation analysis of CD4 and CD8 counts between pre and post-infection stages. Pair 1 (CD4 pre and CD4 post) shows a moderate positive correlation ($r = 0.419$) with a significant p-value of 0.007, indicating that higher CD4 counts pre-infection are associated with relatively higher CD4 counts post-infection. However, Pair 2 (CD8 pre and CD8 post) exhibits a weaker positive correlation ($r = 0.170$) with a non-significant p-value of 0.295, suggesting a less clear relationship between CD8 counts pre and post-infection. The results of HCV infection have a more pronounced impact on CD4 cell counts compared to CD8 cell counts (Figure 1). The gradual decrease in CD4 counts over time in subfigure A could indicate the potential effect of HCV infection on the depletion of CD4 T cells, which are crucial for immune response. The relatively stable CD8 counts shown in subfigure B may suggest a different response or preservation of CD8 T cell levels during the observed time period.

CONCLUSIONS

In conclusion, both CD4 and CD8 T cells play important roles in the immune response against HCV infection.

The moderate positive correlation observed between CD4 counts pre and post-infection indicates that higher CD4 counts prior to infection may contribute to relatively higher CD4 counts after infection. However, the relationship between CD8 counts pre and post-infection is less clear.

RECOMMENDATION

It is important to consider that the functional characteristics of T cells and other factors, such as the CD4/CD8 ratio, also influence immune health and response to HCV infection. Additionally, the observed decrease in CD4 counts and potential preservation of CD8 counts over time highlight the complex dynamics of T cell responses during HCV infection.

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