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Research Article
Level of Interferon-gamma and Interleukin-12 in Several Genotypes of HCV Infections

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Abstract

Background and Objective: The genotypes of HCV display different geographic distribution in worldwide, also as a marker of immune response to antiviral therapy and serves as a guideline for the duration of therapy. The outcome of HCV infection is determined by the host immune response, eradication of HCV depending on rapid induction of immunity. The failure to produce an effective immune response during acute infection is a key factor in the development of chronic hepatitis. This study aimed to investigate the correlation between genotype specific region of HCV in West Sumatra toward the level of gamma (INF-γ) and interleukin-12 (IL-12). **Materials and Methods:** This study combined the molecular examination for determining the genotype of hepatitis C virus and ELISA method for investigating the level of INF-γ and IL-12 in blood serum of patient with HCV infection. The data were analyzed by using Student t test. **Results:** A total of 75 samples, only 53 samples were successfully sequenced in the region of NS5B. The result of study found three different genotypes of HCV (Genotype 1, 2 and 3). The highest frequency of HCV genotype was Genotype-1 (N = 39, 73.58%) and subtype-1a was the most HCV subtype was frequently found. The average of IFN-γ level in HCV genotype 1, 2 and 3 was 10.85, 8.75 and 10.56 pg mL^{-1}, respectively. The average of IL-12 level was 4.08, 5.00 and 3.64 pg mL^{-1}, respectively. This study found that there were no significant differences of IFN-γ and IL-12 levels in HCV genotype 1, 2 and 3. **Conclusion:** The level of IFN-γ and IL-12 did not differ significantly in HCV genotype 1, 2 and 3. HCV genotype-1 was the most genotype found in all sample.

Key words: HCV, interferon-gamma, interleukin-12, genotype, subtype 1a

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

One microliter of amplicon was used for second-round PCR using Go *Taq*Green (Promega, USA). The following cycling parameters were used for 35 cycles. The amplified products were separated by electrophoresis on 2% agarose gel stained with ethidium bromide. The gel was visualized under an ultraviolet trans-illuminator with a 100-base pair (bp) ladder (Promega, USA) and photographed.

DNA purification and sequencing: Amplicon was purified by using the QIAquick PCR purification kit (Qiagen, USA) and visualized by ethidium bromide 2% agarose gel electrophoresis for last verification. Samples were sent to Macrogen, Korean Republic for DNA sequencing analysis.

Alignment of sequence target: The sequence of DNA was edited using Geneious's version R 7.

Identification of IFN-γ and Il-12 levels: The IFN-γ and Il-12 levels were identified based on ELISA sandwich. Briefly, 100 µL blood serum were transferred into wells of microtiter with anti-HCVc antibody which were already coated in plate, incubated for 2 h and wash five times with 250 µL wash buffer. About 100 µL FITC-Conjugated anti HCHcAg were added to each well, incubated and wash, 100 µL HRP-conjugated anti-FITC monoclonal antibody was added into wells. About 100 µL substrate solution was added and stopped the enzyme reaction by adding the stop solution. The absorbance of each well was measured using ELISA reader at 450 nm (R and D systems).

Statistical analysis: Data were analyzed using SPSS ver. 15.0 software, the frequency of samples was presented as means. Data were compared using Student t-test. The p-values<0.05 were considered statistically significant.

RESULTS

In this study, a total of 75 samples, 53 samples (70 .67%) showed positive HCV after amplification. The HCV genotyping using NS5B sequencing obtained good quality of DNA. Result of electrophoresis showed the product of DNA fragment was 449 bp in position (Fig. 1).

A total of 53 samples with positive HCV, only 50 samples could be grouped individually into genotype-1, 2 and 3 it is shown in Table 1. Most of samples was predominantly included into genotype-1 (73.58%), followed by genotype-3 and genotype-2 (16.98 and 3.77%, respectively). Genotype-1 consist of three subtypes (1a, 1b, 1c), genotype-2 only has

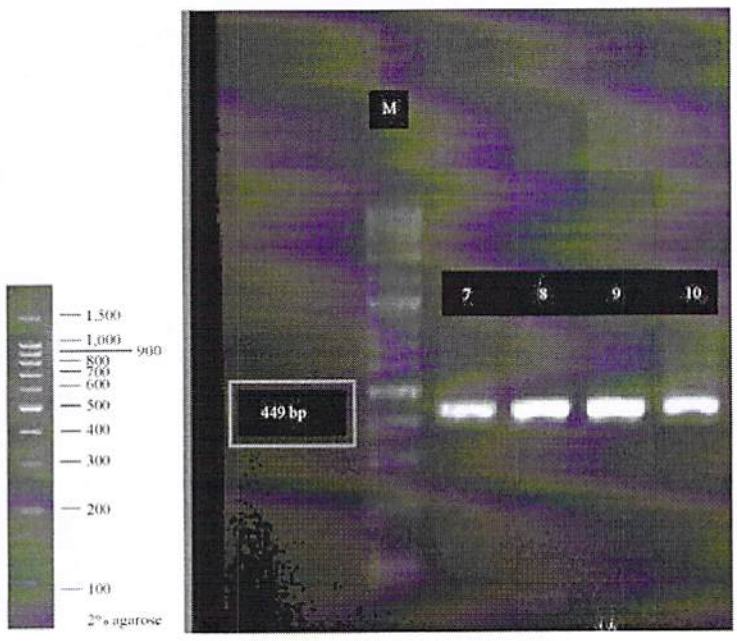

Fig. 1: Electrophoresis result of NS5B PCR products on 2% agarose gel. The amplified DNA band size was 449 bp (M: 100 bp DNA ladder, 7-10: Samples)

The failure of body to produce an effective immune response during acute infection is a key factor in the development of chronic hepatitis. In patients with chronic hepatitis C, acquired CD4 T cell and CD8 T cell decline. Inefficient Th1 cell response was marked by the loss of secretory capacity of IFN-γ caused by wrong differentiation of CD4.

CONCLUSION

The IFN-γ and IL-12 levels varied between three genotypes (Genotype 1, 2 and 3), but the level of IFN-γ and IL-12 were not statistically significant to every genotype of HCV. The results indicate that the HCV genotypes are not the predictor to determine the severe of HCV.

SIGNIFICANCE STATEMENT

Study on genotypes of HCV toward the level of IFN-γ and IL-12 was performed to continue the previous study about the distribution of HCV genotypes in West Sumatra. The HCV are divided into six genotypes distributed largely in worldwide. Most of genotype is spreaded in specific region. The identification of HCV genotypes is important for patient management, determination of therapy duration, response to antiviral and geographic distribution. NS5N is a conservative region to detect HCV genotype. This study found that HCV genotype-1 was the most prevalent genotype than genotype 2 and 3, with 1a as prevalent subgenotype. But, there were no significant differences in IFN-γ and IL-12 levels between three genotypes.

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