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Estimate Viral RNA Of Hepatitis C Of B-Thalassemia Patients In Nineveh Province

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Abstract: Hepatitis C infection is the main occasional agent of chronic liver disease and might lead to cirrhosis and hepatocellular carcinoma. β-thalassemia and hemodialysis patients are most susceptible to HCV as a result of the continuous blood transfusion. Aim of the study: This study aimed to estimate the quantitative of HCV-RNA in the serum of β-thalassemia patients in Nineveh province using RT-PCR in the GeneXpert system. Methods: We detected anti-HCV using ELISA. Determine the viral RNA using Xpert® HCV viral load technique. Result: The positive detection result of anti-HCV is 48 out of a total of 752 thalassemia patients. Only 12 out of 48 enrolled HCV Ct value with a rate of 1.59% of the total β- thalassemia patients. Conclusions: The prevalence of HCV in thalassemic patients in Nineveh province is less than those found in the other Iraqi provinces or adjacent countries. The routine using of immunobiological and biochemical tests for hepatic enzymes should be the routine pursuit in thalassemia and dialysis clinical centers.

Keywords: HCV, Xpert, antibody, thalassemia, RT-PCR.

1. INTRODUCTION:

β-thalassemia (Cooley's anemia)is the most health effective type of thalassemia due to it requires blood transfusion for patients continually. Hepatitis C virus (HCV) causes a contagious and major public health in the world. Hepatitis C virus relates to the *Flaviviridae* family known as a major occasional agent for both active and chronic hepatic injuries which may lead to cirrhosis, and hepatocellular carcinoma [1]. Concerted risk factors for HCV infection are blood transfusions specifically in hemodialysis and thalassemia patients. It also can be transmitted through unscreened donors in addition to injection drug use. Although vertical and sexual transmissions of the HCV have been declared, a large number of the virus carriers have not been identified yet [2]. The prevalence of HCV infection ranges in dialysis and thalassemia patients from 3- 23% compared with the general population in which the ratio reaches 1-3%. This high ratio relates to the truth that these patients have peril factors through receipt of blood transfusions or the use of illicit intravenous drugs [3]. The acute process of HCV is usually asymptomatic and any symptoms abate within a few weeks. The rate of acute cases between 60-80% might develop to chronic hepatitis and only 20-30% of chronic patients may evolve to hepatic cirrhosis. Currently, there is no specific vaccine or medicines could be approved to prohibit replication of the viral RNA or to eliminate the infections [4].

The common and classic test used to detect HCV infection by demonstrating the IgM antibodies in the patient's serum by using enzyme-linked immunosorbent assay (ELISA). This immunoassay utilizes both recombinant and synthetic antigens to the disclosure of the viral antibodies in human serum or plasma. These antigens coincided with the conserved

epitopes of the virus. For further disclosure measures, the quantitative of viral RNA is used for various approaches including real-time polymerase chain reaction, transcription-mediated amplification, multiplex PCR, and branched-chain DNA (bDNA) tests [5,6]. Each commercial test has a lower limit of detection (LLOD) and a lower limit of quantification (LLOQ). Therefore the patient's results may be different based on the application of assay. HCV-RNA test must be less than LLOQ of 25 IU/ml if it is used to estimate treatment response [7].

This study aims to estimate the HCV-RNA of β-thalassemia patients using GeneXpert in Nineveh province. This system is intended for estimating the quantitation of the viral RNA in human serum or plasma. GeneXpert is an automated RT-PCR through applied fluorescence dye to detect the viral RNA. This method measures levels at a baseline during treatment or non-sustained cases of therapy.

2. MATERIAL AND METHODS:

This study was conducted at the thalassemia section in Ibn-Alatheer Hospital in Mosul city, from July 2018 to February 2020. The GeneXpert tests were held at Nineveh Tb Centre. A total of 752 patients participated in the current study (458 male 60.9% and 294 female 39.09%) aged from (2-35y) with a mean (14.52), Std. error (±1.20), and Std. deviation (±12.03527). Blood samples were collected from the patients and the serum was separated by centrifugation and kept frozen for the next analysis.

Determine the HCV antibodies using ELISA: The immunoassay utilizes both recombinant and synthetic HCV antigens to detect antibodies of HCV in human blood. The existence of anti-HCV in serum was detected using a commercial kit purchased from (DIALAB- Austria) and (BIOLABO, France) upon the manufacturer's instruction for all suspected patients. The positive results with cut-off > 1 were repeated twice for confirmation, then they were kept freezing for detection of the HCV-RNA. Specimens with absorbance equal or greater than cut-off value were realized as initially reactive and initially re-tested to exclude the possibility of a cross-reaction.

The principle of Xpert® HCV Viral Load technique and application: The GeneXpert is an automate system device which integrates sample purification, nucleic acid amplification, and detection of the target sequence. GeneXpert cartouche contains the reagents and runs the RT-PCR process. So, the cartridge is self-contained and the sample contamination is minimized. Determination of the RNA reagents and two internal controls inside the cartouche is used for estimation quantitation of the HCV-RNA. The probe check control (PCC) proves reagent rehydration. The PCR tube is filling with probe integrity and dye stability inside the cartouche. A 1 mL of serum was filled into the sample chamber of the test cartridge. Then, the cartridge was loaded on the conveyor belt into the GeneXpert device.

Descriptive controls and samples: Each test includes sample volume adequacy (SVA), internal quantitative standard high and Low (IQS-H and IQS-L), and probe check control (PCC) pass if it meets the validated acceptance criteria. The SVA achieves the correct volume of the sample when it has been added to the chamber. The two laminated RNA (IQS-H and IQS-L) are constructed in the form of dry beads that go through the whole assay process that calibrated as an international standard for HCV. The automated system measures the fluorescence signal from the probes to monitor bead rehydration, then the reaction tube is filling, according to the dye stability, and probe integrity. The results were interpreted automatically. The total duration RT-PCR for each sample takes exactly 105 min.

Statistical analysis: The data analysis was performed using SPSS software version 25.0 specifically for the calculation of the mean, standard deviations, standard error, and to assess the significant P-value.

3. RESULTS:

Based on the statistical analysis, there is no significant value between infections and genders at $P > 0.01$. The current study is scored high significant value at Pearson correlation between age parameter and HCV Ct cycle with $P < 0.05$. A total of 48 (6.38%) out of 752 total thalassemia patients obtained positive results for anti-HCV using the ELISA test with the titer > 1, table (1). Scattered dots and spline graphs are illustrated distribution ELISA titers with the samples, fig. (1). The positive results were tested again for confirmation using the same inspection. Despite of the high sensitivity and specificity of the enzyme immunoassay test for detecting the antibodies, it is still potential having both false-positive and false-negative results.

The anti-HCV positive result for 25 males and 23 females were enrolled for determination of RNA by GeneXpert. Only 12 (25%) patients out of 48 were registered positive of HCV-RNA in their serum. Analyte results for all samples described in table (1). The cycle threshold value for all positive results is > 1, table (1). Scattered dots and spline graphs are illustrated distribution HCV cycle threshold with the samples, fig. (2). The results are explicated automatically by the system through measured fluorescent signals and established calculation algorithms. Scores are clearly shown in the view results window, fig. (3). When HCV undetected (0.0) and the standard control IQS-H and IQS-L passed, the result is negative as shown in fig. (3 A1 and A2). When the HCV-RNA was detected below the quantitative range < 10 IU/mL and < log 1.0 with pass control, a positive detection emerges as shown in fig.(3 B1 and B2). The current study recorded HCV-RNA within the linear range setting of the assay and the endpoint above the minimum (i.e. > 10 IU/mL and < 30 IU/mL, > log 1.0) with the control and probe cheque pass as shown in fig. (3 C1 and C2). We also detected the HCV-RNA above the quantitative range of the assay > 1E08 IU/mL with the probe and control standard cheque pass as in fig. (3 D1 and D2).

The negative HCV-RNA results of 26 (75%) patients who hold the positive results of anti-HCV are non-deterministic due to the possibility that they might have been infected previously and then they have recovered. Finally, the ratio of the positive HCV-RNA out of the total number of thalassemia patients is 1.59%.

4. DISCUSSION:

It has been predestined that approximately 185 million people have been infected with hepatitis C universally and over 80% of them live in low and middle-income countries. It is known that the riskiest type of thalassemia is the beta major in which the HCV is transmitted during the blood transfusion process [8]. Guidelines for the management and treatment of HCV depend on the estimation of quantitative viral RNA testing before starting antiviral therapy and after the conclusion of treatment [9]. Quantitation of the viral RNA has assured useful for providing a metric to evaluate the effectiveness of the antiviral response to treatment. The main objective of treatment is sustained virologic response (SVR) which is clarified as the undetectable of the viral RNA by the sensitive test for 12 or 24 weeks after the end of remediation depending on the anti-HCV therapy [10]. It would not be suitable to frequently inspected viral RNA for a patient who is recently on HCV treatment or using the test to determine the severity of infection or the patient under the developing serious liver

disease. To consider a response, the quantity of the viral RNA by IU/ml of blood ought to be measured before and within the course of treatment [11].

Iraq is one of the countries in the Middle East that has had an increased incidence of Hepatitis C infections over the past few years. The increasing incidence can be observed in hemodialysis and thalassemia patients, due to the lack of health awareness among individuals, the failure of follow-up of new cases, the deficiency of appropriate treatment in addition to the failure invention suitable vaccine. Our previous study determined a high ratio (3.46%) out of hemodialysis patients infected with HCV. This study disclosed that chemokine-10 level in the serum plays a crucial role in distinguishing between chronic hepatitis C patients who have continuously or discontinuously taken drugs [12]. For instance, the prevalence of HCV in Egypt is around 75% among thalassemia patients. The dispersal of hepatitis C in the blood donor population was 14.5% in Egypt while it reaches only (1.78%) of the total donor population in India [13, 14]. Iran has recorded 28.1% of Hepatitis C patients being thalassemia patients. A study exposed 24.2% of them were positive for the anti-HCV test. The wide prevalence range of the HCV infections in Iran provinces was between 16 - 64% [15]. Moreover, a study estimated 8% of HCV among thalassemia patients in Isfahan-Iran [16]. It is known that blood transfusion is the main risk factor for the transmission of the hepatitis C virus amongst beta-thalassemia patients [17].

It is important to pay attention that the formal screening of blood donors and recipients is an intact manner for detracting the infections. Therefore, more efforts must be applied to improve blood transfusion integrity with high specificity and sensitivity immunoserological tests. Although some of the serological tests may accord contentious results [18], the positive detection of HCV-RNA may emerge as a negative result of anti-HCV with immunocompromised patients [19, 20]. If the given of the availability of safe drug and the frequent follow-up of thalassemia patients in the specific units could be on the road towards the global HCV elimination in each country [21, 22].

Although the dispersal of hepatitis C among beta-thalassemia patients increased in high ratio, our data exhibited a reduction of infection ratio and this is an important indicator compared to the remainder of the studies that have elaborated in this field

5. CONCLUSIONS:

In conclusion, thalassemia patients are remain increasing and at risk of acquiring HCV infection leading to progression to liver cirrhosis and hepatocellular cancer. The prevalence of HCV in thalassemic patients in Nineveh province is less than those found in the other Iraqi provinces and adjacent countries. However, the following simple measures including executed general asepsis rules, using careful disinfection, sterilization of devices, routine testing of patients, and estimation of biochemical tests for liver function enzymes must be the routine practice in thalassemia and dialysis clinical centers.

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Conflict of interest:

The authors declare that there is no conflict of interest in this study.

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Table 1: Full results outcome of ELISA titer and GeneExpert. HCV Ct: Cycle threshold value 0.0 < 1 (-ev), (+ev HCV) > 1. IQS-H: Internal quantitative standard high. IQS-L: Internal quantitative standard low.

Sample no.	Sex	ELISA titer	HCV Ct	IQS-H	IQS-L	Sample no.	Sex	ELISA titer	HCV Ct	IQS-H	IQS-L
1	F	1.6	28.2	23.1	33.7	25	M	2.1	20.7	19.9	30.4
2	F	1.3	0.0	22	33.3	26	F	2.3	0.0	22.4	32.8
3	F	1.1	0.0	22.2	33.1	27	M	2.5	0.0	22.5	34.2
4	M	2.3	0.0	22.2	32.9	28	F	2.6	0.0	22.7	31.5
5	M	3.4	0.0	22.4	33.1	29	M	4.6	0.0	21.9	32.4
6	F	4.8	0.0	22.1	32.8	30	M	5.9	0.0	22.2	32.9
7	M	6.2	0.0	21.8	32.4	31	F	6.6	37.2	22.4	32.6
8	M	4.2	0.0	22.4	33.3	32	M	14.7	0.0	21.9	32.5
9	F	5.3	0.0	21.9	32.5	33	M	14.8	0.0	22.1	32.4
10	F	3.7	0.0	22.5	33.2	34	M	14.9	0.0	22.4	33.1
11	F	13.5	0.0	22.2	32.9	35	F	15.1	22	22.2	32.9

12	M	15.4	0.0	22	33	36	M	15.4	0.0	22	33.3
13	F	1.2	43	22.4	33.5	37	M	15.6	0.0	22.5	32.1
14	M	6.6	0.0	23	33.5	38	F	15.7	0.0	22.6	32.9
15	M	4.1	0.0	22.5	32.8	39	M	15.8	22.1	23.1	33.6
16	F	15.4	0.0	22.1	33.1	40	F	15.8	0.0	22.4	33.2
17	M	13.2	0.0	22.5	33.3	41	F	15.5	0.0	22.4	33.1
18	F	15.8	0.0	22.3	32.6	42	M	15.6	0.0	22.2	33.3
19	M	1.6	26.5	22.5	33	43	M	6.8	15.8	22.4	32.8
20	F	12.3	20.8	22.4	32.9	44	F	15.1	0.0	22.2	32.4
21	M	13.6	0.0	22.6	33.1	45	M	15.6	20.2	23.1	33.7
22	F	4.4	0.0	22.3	32.9	46	M	3.4	0.0	22.2	33.1
23	F	1.6	0.0	22.4	33.3	47	F	11.3	20.2	20.1	33.7
24	F	2.6	34.6	21.9	34	48	M	5.3	0.0	23.3	32.1

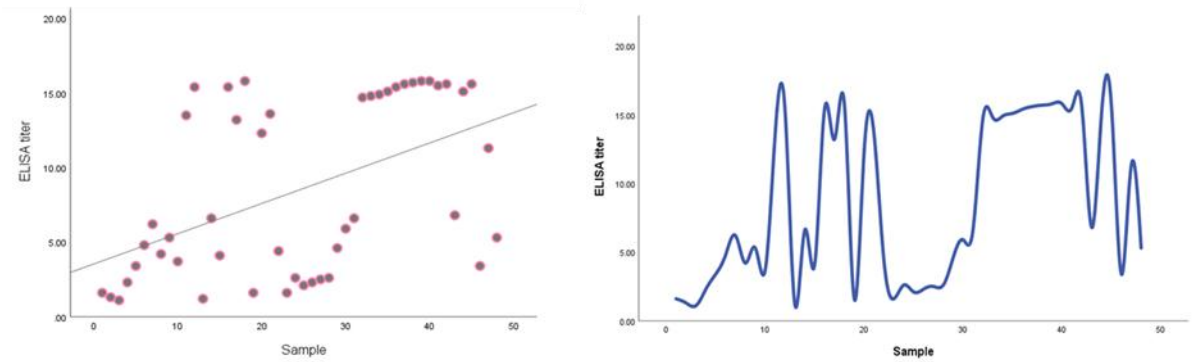

Figure 1: Distribution of ELISA titer positive results with the meant value in scattered dots and spline graphs.

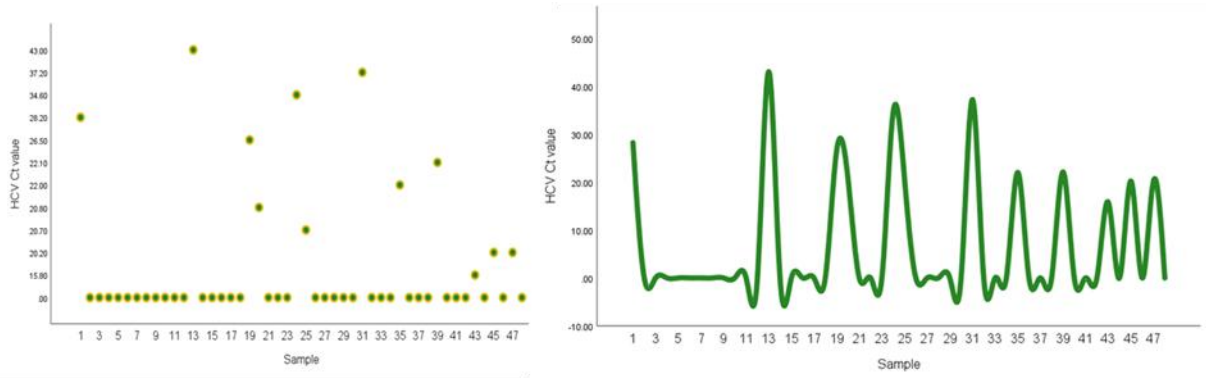

Figure 2: Distribution of HCV Ct value negative and positive results with the meant value in scattered dots and spline graphs.

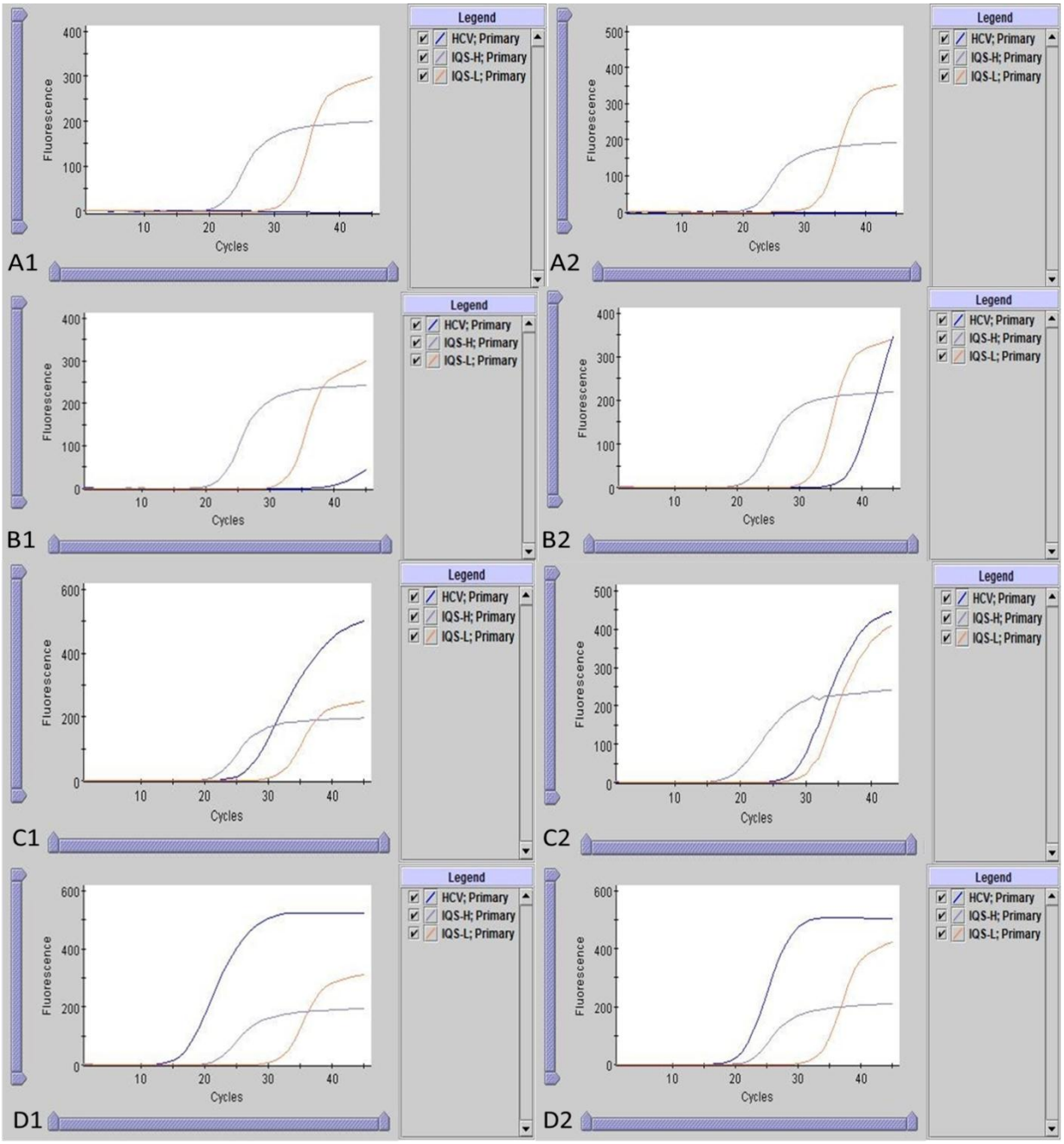

Figure 3: Interpretation and layout of the results from GeneExpert. IQS-H: Internal quantitative standard high. IQS-L: Internal quantitative standard low. A1& A2: HCV-RNA not detected (-ve). B1 & B2: HCV-RNA detected < 10 1U/mL (log 1.00). C1 & C2: HCV-RNA detected 500 1U/mL (log 2.74). D1 & D2: HCV-RNA detected > 1.00E08 1U/mL (log 8.00).