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Serological and Molecular Study for Hepatitis C Virus in Thalassemia Patients in Wasit Province/Iraq

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ABSTRACT

Thalassemias are a heterogeneous group of hemoglobin synthesis genetic disorders resulting from a decreased production rate of one hemoglobin chain or more. This study was conducted on151 samples divided into three groups:

β- Thalassemic patients with hepatitis C (66) patient's, 35 patients' non thalassemia patients with the hepatitis C and 50 healthy control.

This study included, Serum iron profile along with liver functions were determined. Real-Time qPCR was used to identify and assessment of viral loads in the blood of HCV patients. In the current study, there was positive Correlation between hepatitis C status and serum ferritin level in both groups of patients when compared whit healthy controls, the levels of the serum ALT in the patients of the CHC have been greater. There has been as well a considerable association between the ALT levels and the HCV RNA titer. We conclude in this study, high prevalence of 1a genotype in thalassemic patients (60.7%) then 1b genotype (21.4%). followed by 4 genotype that was (17.9%). While in non thalassemic patients with HCV also high prevalence of 1a genotype (48.6%) then 1b genotype (22.9%) followed by 4 genotype that was (20.0%) and 3a genotype that was (8.6%).

Keywords: Thalassemia, genotyping, hepatitis C virus,viral Load.

Introduction

Thalassemia is inherited blood disorders; it is spread in the Mediterranean region. Middle Eastern, Southeast Asian and African have the ability to carrying thalassemia genes (Bhandari *et al*, 2018).The virus of Hepatitis C is responsible for more than 85% of the transfusion-related cases of hepatitis. The possibility of the progression of the infection into chronic case becomes over 50%, which will result in hepatocellular carcinoma or cirrhosis in 25%. Almost all world countries had established their own data base regarding HCV. It has been projected that over 170 million persons have got the infection all over the world. In the industrialized countries, the prevalence of the Hepatitis C virus is often 1 to 2% in general populations and less than 0.50% in the blood donors (Amiri *et al*, 2005).. HCV can be considered as the most widespread post-transfusion hepatitis (PTH) cause as well as end-stage liver disease in numerous regions. The aims of this study

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identify of Hepatitis C virus amongst multiple-transfused thalassemia patients and to determine the most prevalent genotype /subtype for this virus

Materials and Methods

A total 66 specimens of blood samples have been collected from subjects experiencing thalassemia who have been admitted in Thalassemia hematology center in Al Kut women &children Hospital in Wasit province / Iraq, during the period from September 2019 to February 2020. The patient's age were ranged from 3 to ≥ 51 years. The patient was diagnosed at the thalassemia syndrome patient was regularly attending the hematology clinic in Thalassemia hematology center in Al Kut women &children Hospital ,either for transfusion and chelation and follow up of Hb level and iron status. Also, the diagnosis that is related to β-thalassemia has been on the basis of hemoglobin electrophoresis, hematological indices, clinical presentation, as well as the iron overload. An ethical verbal and written consent were taken from patients. Thirty-five specimens were collected from apparently stable control group, who had no history of thalassemia but infected with hepatitis C virusand 50 healthy controls. Blood Collection. Five milliliter of the venous blood acquired through vein puncture from each one of the subjected (controls and cases) included in this work, the blood has been almost equally divided into (2ml) EDTA tubes, also (3ml) in the serum tubes (the samples of blood have been collected into EDTA tubes and plain plastic tubes were prepared and labeled). (3) ml blood sample obtained from each one was clot at room temperature for one hour, and then the centrifugation has been conducted for a period of five minutes, at (4000) rpm for the purpose of separating the serum.

Hematological analysis. CBC, complete system related to reagents of control as well as calibrator, Cell-DYN Ruby Hematology Abbott / USA.

Biochemical analysis.Liver function tests, parameters (Serum ALT, AST, ALP and TSB), by Reflotron.

Iron parameter Iron and TIBC (Total Iron Binding Capacity) have been evaluated with the use of commercial kit (Colorimetric technique) generated via Liner Ferrozine (ASPAN).

VIDAS Ferritin Required information has been obtained from records of patients.ELISA for detecting antibodies to HCV.

Viral RNA Extraction**.** With regard to Ribo Virus, the RNA viruses have been quickly and effectively lysed through lysis buffer RAV-1 that has been highly concentrated solution that is related to the GITC. Also, the Lysis buffer as well as the ethanol creating adequate conditions of binding the nucleic acids into the membrane of the silica in columns of theRibo Virus

Viral Load. The real-time PCR can be defined as a homogenous very sensitive assay combining the amplification with the fluorescence-based online discovery of the relevant nucleic acid (i.e. target, template), it became a main technique in the area of the molecular diagnostics.

HCV Genotype Plus Real-TM .ThePCR kit of AmpliSens® *HCV*-genotype-FRT

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Statistical Analyses: The statistical analysis has been performed with the use of the SPSS software, v20.0 (IBM Corporation, US); the data have been demonstrated as percentiles for the non-numerical data and as the median for the numerical data. P values <0.05 have been considered to have statistical significance.

Results and Discussion

This study included 101 patients divided to 66 β-thalassemic patients with HCV, 35 non-thalassemic patients with HCV as well as 50 healthy control .The β- thalassemic patients with HCV were 52 patients' β- thalassemia major Frequency (78.8) and 14 patients' β- thalassemia intermedia frequency (21.2). In this study, the ages ranged from 3 years to 67 years, in β- thalassemic patients 36 patients less than 25 years the frequency (54.5) and 30 patients more or equal to 25 years the frequency (45.5). The mean age for β- thalassemic patients with hepatitis C was (23.9±9.4) years. non thalassemic patients with hepatitis C the 7 patients less than 25 years the frequency (20) and 28 patients more or equal to 25 years the frequency (80) .The mean age for non β- thalassemic patients with hepatitis C was(37.54 ± 1.5)year's. In healthy control 32 individual less than 25 years the frequency (64) and 18 individual more or equal to 25 years the frequency (36) . The mean age for healthy control was (29.7± 8.59) years. The distribution of patients according to gender in β- thalassemic patients appear 34 male with frequency (51.5) and 32 female with frequency (48.5).While in non β- thalassemic patients with hepatitis C were 14 male with frequency (40) and 21 female with frequency (60).In healthy control was 29 male with frequency (58) and 21 female with frequency(42).The viral load in β- thalassemic patients with HCV were 28 patients positive with frequency (42.4) and 38 patients negative with frequency (57.6) . While in non β- thalassemic patients with HCV were all patients positive. Ferritin level in β- thalassemic patients with HCV was 28 patients less than 3000 ng/dl with frequency (42.4) and 38 patients more or equal 3000 ng/dl with frequency (57.6). While in non β- thalassemic patients with hepatitis C was 27 patients less than 3000 ng/dl with frequency (77.14) and 8 patients more or equal 3000 ng/dl with frequency (22.86).While in healthy control was all individual less than 3000 ng/dl.

Laboratory findings of this study include serum iron, Hb, TBIC, AST, serum ferritin, ALT, ALP and TSB show a table (1).

Table (1): Laboratory findings of study.

Mean ±SD				
Item	β T with HCV no.66	Non β T with HCV no.35	Healthy control no.50	P-value
Hbμg/dl	9.32±1.4	13.6±0.8	13.4±0.9	<0.001
IRON/μg/dl	197.86±6.2	112.89±1.6	84.6 ±2944	<0.001
TIBC/μg/dl	208.82±5.7	257.54±4.7	321 ±53.19	<0.001

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S.F ng/ml	5630.93±5.5	1805.89±1.9	127.7 ±40.29	<0.001
AST u/L	58.2±4.1	55.95±37.44	18.09±10.24	<0.001
ALT u/L	67.6±5.9	63.97±44.49	19.14±10.02	<0.001
ALP u/L	224.82±1.1	178.07±8.8	73.2±17.57	<0.001
TSB mg/dL	2.3±2.0	1.9±0.98	0.88±1.2	<0.001

Extreme anemic presentations have been specified in the β-thalassemia patients. Low levels of (RBCs, Hb, HCT, MCV, MCH, and MCHC) were significant decreased ($P < 0.001$) expected in thalassemic patients because β-thalassemia is an inherited Hb synthesis disorder resulting in severe anemia (Nienhuis and Nathan, 2012). This outcome was similar to other research (Sadoon and Bashir, 2010)There was a decrease in patient's hemoglobin levels comparing to the recorded level in controls, with significant differences ($P <0.05$) respectively, so they had needed blood transfusion which was a primary reason for iron overload(Fadhil *et al*, 2015)..This the results showed all patients have severe anemia as compared to the controls except that for HCV group with no significant difference in hematological parameter (CBC) ($P>0.05$) compared to the control. This result agrees with (Streiff, Mehta and Thomas, 2002) who reported HCV infection is associated with low neutrophil and platelet counts, but not anemia, in the general population of the United States.

Iron parameters included (Serum Ferritin, TBIC, and Serum Iron) were detected in β- thalassemic patients with HCV and non β-thalassemic patients with HCV and controls. The difference has been extremely significant (P less than 0.001) in patients in the case when put to comparison with the healthy controls, with a mean ±SD as shown in table (3-2).The Serum iron (mg/dl) and serum ferritin (ng/ml) a significant increased ($P < 0.001$) in the patient's groups in the case when put to comparison with the healthy controls. At the same time, Serum TIBC (mg/dl) was a significant decreased ($P < 0.001$) in patient's groups in the case when put to comparison with the healthy controls, as shown in table (2).

Table2: The serum Iron, TIBC and Ferritin of the studied groups.

Mean ±SD				
Item	β T with HCV no.66	Non β T with HCV no.35	Healthy control no.50	P-value
IRON/µg/dl	197.86±6.2	112.89±1.6	84.6 ±2944	<0.001
TIBC/µg/dl	208.82±5.7	257.54±4.7	321 ±53.19	<0.001
S.F ng/ml	5630.93±5.5	1805.89±1.9	127.7 ±40.29	<0.001

TIBC: Total iron binding capacity, **S.F:** Serum ferritin.

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In the table shown the serum iron level was a significant increase in thalassemia patients when compared healthy controls. This result that agreement with (Kaddah *et al.*, 2011)who reported serum iron level in TM which is significantly higher in TM compared to TI and in both groups compared to control. That agreement with (Widyastiti *et al.*, 2018) study, also he explains the blood transfusion of thalassemia patients lead to increase of serum iron. Additionally, iron test resulted in decrease highly significant ($p< 0.001$) with control, that agreement with (Inthawong *et al.*, 2015), also he discusses the aggregation of iron in thalassemia patients that lead to increase iron concentration.In iron's excess or overload, there will be increase in the ferritin's iron composition(Al-Hakeim and Al-Hakany, 2013) and this may be the most important cause for the elevation of serum ferritin. In the current study, serum ferritin has been considerably high in HCV patients in comparison to control group. This is in agreement with the findings of (Fujita *et al.*, 2008) and (El Lehleh *et al.*, 2017) .whose found that serum ferritin has been significantly high in CHC group when compared to control group (*P* less than 0.001). In one study, the TIBC level decreased significantly in the β-thalassemia group in comparison to those in other control group. Used the increase in serum iron level and decreased TIBC level in the diagnosis of β-thalassemia in children with anemia (Huang *et al.*, 2010).There was a high significant difference of AST, ALT, ALP, and TSB detected in four groups of patients and controls (P less than 0.001) when put to comparison with the healthy controls, as shown in the table (3).

Table3: Liver functions results of the studied groups.

Mean ±SD				
Item	β T with HCV no.66	Non β T with HCV no.35	Healthy control no.50	P-value
AST u/L	58.2±4.1	55.95±37.44	18.09±10.24	<0.001
ALT u/L	67.6±5.9	63.97±44.49	19.14±10.02	<0.001
ALP u/L	224.82±1.1	178.07±8.8	73.2±17.57	<0.001
TSB mg/dL	2.3±2.0	1.9±0.98	0.88±1.2	<0.001

AST: Aspartate aminotransferase, **ALT:** Alanine aminotransferase, **ALP: Alkaline** Phosphatase, TSB**:** total serum bilirubin.

Complications of iron overload include endocrinopathy, heart and liver disease and complications of chelation therapy (Ferrara *et al.*, 2004). Since the liver is the first organ which is affected via iron overload in thalassemia. A higher level of serum AST, ALT, ALP and TSB in β-thalassemia patients indicate an abnormal liver function in the presented work showed that the iron over load affects liver function in thalassemic patients. These finding are in agreement with the finding of (Salih and AL-Mosawy, 2016) who reported Iron overload with an average ferritin level of approximately 3162.7 ng/ml, in relation to serum TSB, GPT, GOT and ALP levels of approximately 76.2, 31, 42.9 and 21.4% of total patients, respectively. The level of serum AST, ALT, ALP and TSB in β-thalassemia

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with HCV patients increased significantly P<0.001in compared to controls. Our result is agreement with the finding of (Wanachiwanawin *et al.*, 2003).

The results revealed that all thalassemic patients was positive by ELISA technique, while 28 (42.4%) were gave positive results for HCV RNA, this divided according type of thalassemia to 21(31.8%) thalassemia major and 7 (10.6%) thalassemia intermedia, and 38 (57.6%) were gave negative results for HCV RNA divided to 31(47%) thalassemia major and 7 (10.6%) thalassemia intermedia table (4),while all non thalassemic patients with HCV were positive results for HCV RNA.

Table (4): Correlation between hepatitis C status and type of thalassemia

Diagnosis	Negative (%)	Positive (%)	Total (%)	P value
Thalassemia major	31(47)	21(31.8)	52(78.8)	0.3
Thalassemia intermedia	7(10.6)	7(10.6)	14(21.2)	

It has been typically verified predication that in the patients experiencing CHC, high HCV-RNA as well as serum ALT levels indicating the existence of active HCV replications in the liver and, therefore, liver injury indicates clinical risk. The serum ALT levels in patients experiencing CHC have been high. There is a considerable relation between ALT levels and HCV RNA titer. These results are in accordance with (Rukiye and Sezgin, 2016). The results are in agreement with Shahraki *et al.*, 2010) in which they indicated a relation between the existence of HCV RNA PCR as well as the abnormal liver tests

In our study the Correlation between hepatitis C status as well as serum ferritin levels in β- thalassemic patients with HCV was 13 (19.7%) patients positive HCV, 15(22.7%) patients negative HCV with serum ferritin level less than 3000 ng/dl and 15(22.7%) patients positive HCV, 23(34.8%) patients negative HCV with serum ferritin level more or equal to 3000 ng/dl. While innon-thalassemic patients experiencing HCV, all patients positive HCV 27 (77,14%) with serum ferritin levels not more than 3000 ng/dl and 8(22.86%) with serum ferritin level more or equal to 3000 ng/dl.In Heathy controls the serum ferritin level was normal table (5).

Table (5): Correlation between hepatitis C status and serum ferritin level

β- thalassemic patients with hepatitis C			
Serum ferritin ng/dl	Negative (%)	Positive (%)	Total (%)
less than 3000	15 (22.7)	13 (19.7)	28 (42.4)

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more or equal to 3000	23 (34.8)	15 (22.7)	38 (57.6)
non thalassemic patients with hepatitis C			
less than 3000	/	27(77.14)	27(77.14)
more or equal to 3000	/	8(22.86)	8 (22.86)
Heathy controls			
less than 3000	50	/	50 (100)

In the current study, serum ferritin has been considerably high in HCV patients in comparison to the control group. This is in agreement with the findings of (Fujita *et al.*, 2008)and(El Lehleh *et al.*, 2017) .whose found that serum ferritin has been considerably high in CHC group in comparison to control group (*P* less than 0.001).

The study was conducted on 63 of HCV RNA positive individuals of both sexes gathered comprising as: (28) thalassemic patients; (35) non thalassemic patients with HCV. The age ranged from 6 years to 67 years. The distribution of patients according to sex in β- thalassemic patients appear17 males and appear 11females.While 14 males and 21 females in non thalassemic patients with hepatitis C. The mean age for β- thalassemic patients with hepatitis C was (21.68±5.4) years and non thalassemic patients with hepatitis C was (37.54 ± 1.5). The results showed high prevalence of 1a genotype in thalassemic patients (60.7%) then 1b genotype (21.4%). followed by 4 genotype that was (17.9%) table (6). While in non thalassemic patients with HCV. The results showed also high prevalence of 1a genotype (48.6%) then 1b genotype (22.9%) followed by 4 genotype that was (20.0%) and 3a genotype that was (8.6%) table (7).

Table (6): Hepatitis virus genotype in thalassemic patients**.**

Hepatitis C genotype	Frequency	Percent
1a	17	60.7
1b	6	21.4
4	5	17.9
Total	28	100.0

Table (7): Hepatitis virus genotype in non thalassemic patients.

Hepatitis C genotype	Frequency	Percent

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1a	17	48.6
1b	8	22.9
4	7	20.0
3a	3	8.6
Total	35	100.0

our results were an agreement with (Hassan *et al.*, 2018) in Erbil.Who concluded the most predominant genotype was the G1 (37%) which includes the sub-type 1a (26.1%) and the sub-type 1b (10.3%), followed by the G3, G4, & G2 in a descending order.

Our results were an disagreement (Alwaysi *et al.*, 2018)in Iraq. Who concluded that only genotypes 4 and 1 of the HCV have been found in respectively 54%, 46%, of the patients that are HCV-RNA positive. The differences above in the patterns of the genotypes/sub-types in Iraq can be a result of the fact that the genotype 4 of the HCV can get higher resistance to the treatment and has become under selective therapy pressure, particularly the sub-types with the highest resistance to the therapy have been 4d and 4e that can be explaining the increase in the HCV genotypes 4 over 1 percentage over the time in Iraq (Alwaysi *et al.*, 2018).The our study were an agreement with (Khdeir *et al.*, 2016) in Basra Province/Iraq who reported that genotype 1 is the most common. In conclusion ,the predominant of HCV genotypes in Wasit patients was genotype 1a followed by genotype 1b followed by genotype 4.

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