Study the expression level of beta 2 microglobulin gene on hepatitis C patients before and after treatment with interferon

Mohammed A. Saleh*

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Abstract:

This study has been carried out to evaluate the expression level of beta 2 microglobulin gene on patients infected by hepatitis C virus before and after treatment with interferon. The study included 117 hepatitis C patients comprising as 63 pre-treated patients, the range of age was between 20-65 year with a mean age of 48.12 ± 16.1 and 54 post-treated patients with age range was between 23-63 year with the mean of 46.1 ± 18.1. Also it was found that more than half of patients were located within third and fourth decade i.e. 30-49 year, with a percentage of 52.4% and 55.6 % for pre-treatment and post-treatment patients respectively. Moreover , regarding both groups, males are more than females with the ratio of (3.2:1) among pre-treatment group and 2:1 among post-treatment group. Further , It has been found that the concentration of β2 microglobulin was (3.425±0.943mg/L) among pre-treatment group and (1.860±0.723 mg/L) among post-treatment group with significant correlation (P=0.05). Besides that , in the present study ,It has been found the concentration of β2 microglobulin was decrease after treatment from (3.425±0.943 mg/L) to (1.860±0.723mg/L) which was statistically significant (P=0.05) , Thus β2 microglobulin can be used as a supporting marker of responsiveness to treatment with interferon in hepatitis C patients as well as indicator for monitoring the disease progression.

Key words: beta 2 microglobulin gene , Hepatitis C Virus, Interferon

Introduction:

Hepatitis C virus (HCV) infection has become a major public health problem, with 170 million people considered to be infected worldwide. The disease progresses slowly and a chronic infection develops in 85% of the cases. Among patients with chronic hepatitis, 20 to 30% develop cirrhosis that, once established, carries a poor prognosis, with a high risk of developing hepatocarcinoma [1]. Structural studies of the HCV genome have shown that virus have a positive – strand RNA virus related to flaviviruses family , The high rate of mutation in the RNA genome of this RNA virus may cause the variability of the envelope protein [2,3] HCV has been linked to a blood – borne , e. g. patients receiving organ transplants , blood product , or intravenous drug use , born to an infected mother , and sexual practices [4] . Infection with acute HCV is usually subclinical, but the likelihood of chronic is high. Infection with HCV is most typically diagnosed in the chronic phase of infection [5].

β2 - microglobulin also known as B2M which is a component of MHC class I molecules, which are present on all nucleated cells (excludes red blood cells).In humans, the β2 microglobulin

*Biology Department, College of Science , Diyala University.

protein is encoded by the B2M gene.β2 microglobulin lies lateral to the α3 chain on the cell surface. Unlike α3, β2 has no transmembrane region. Directly above β2 (i.e. away from the cell) lies the α1 chain, which itself is lateral to the α2.β2 microglobulin associates not only with the alpha chain of MHC class I molecules, but also with class I-like molecules such as CD1 and Qa-1 a form of alloantigen [6,7]. Beta-2microglobulin is associated with an activated immune response in HCV infection which may be released by activated lymphocyte (T4/T8 cells), so increasing its level might indicate increasing HCV replication- related cell death .Numerous studies have confirmed an increased beta-2 microglobulin measurement as predictive marker for rates of progression to hepatitis particularly when combined with other direct immunological markers, including CD4+ T cell number[8,9]. Interferon (IFN) most likely has a direct antiviral effect that possible disrupt viral replication .There is an evidence that IFN has a direct anti – viral action via induction of 2,5 A oligoadenylate synthatase and ribonucleose – L (i.e: Interferon has immunomodulating and antiviral effects on hepatitis C virus)[10]. Due to the complexity in studying the human leukocyte antigen (HLA) system, therefore the aim of this study to estimate the alteration in the expression of class I HLA molecules (beta 2 microglobulin gene) indirectly by evaluating serum beta2-microglobulin levels in patients with hepatitis C before and after treatment with interferon (IFN).

Materials and Methods:

Subjects

Patient Study Group

One hundred seventeen (117) patients with hepatitis C- virus (marked with appearance of anti-hepatitis C antibodies in their sera and persist more than 6- months which was detected by using ELISA- technique) comprising as 63 pre-treated patients, the range of age was between (20-65 year) and 54 post-treated patients with age range was between 23-63 year .These patients included 48 males and 15 females as regard to pre-treated patients whereas 36 males and 18 females as regard to post-treated patients .These patients had been clinically diagnosed according to the previous laboratory test and the clinical examination when they were in patients admitted in Gastroenterology and Hepatology Teaching Hospital in Baghdad ,during the period from October 2009 to December 2010.

Samples collection

From each individual included in this study, 5 ml of blood was drawn by vein puncture using disposable syringes. The blood has been placed in plastic disposable tubes, it was left to stand at room temperature (20-25°C) to allow it to clot, then the sera has been separated by centrifugation for 5 minutes, and divided into aliquots (250 µl) and stored at -20°C till examination. Each aliquot of the serum has been used once to avoid thawing and freezing. All sera and reagents were allowed to stand at room temperature before use in the test.

Methods:

1-ELISA for detection of Anti-HCV IgG

Principle

Anti-HCV enzyme immunoassay kit (Biokit, Spain) was a qualitative determination of Abs to HCV (anti-HCV) in human serum or plasma samples. Diluted patient's sample (serum or plasma) has been added to microtiter wells precoated with purified antigen mimicking the core,

NS3, NS4 and NS5 gene segments proteins . These peptides has been shown to react and bind with the predominance classes of anti-HCV Abs present in HCV positive serum.

After incubation, peroxidase-conjugated anti-human IgG Ab has been added to form a detectable complex, and then, substrate-tetramethyl benzidine (TMB) has been added to form a colored complex. The intensity of color has been proportional to the amount of anti-HCV present in the sample, then, the reaction was stopped by the addition of acid and the resulting color intensity can be read spectrophotometrically at 450 nm. The detailed procedure has been carried out as has been suggested in the leaflet supplied with the test kit (Biokit, Spain) [11].

2. Immunoblot Anti-HCV (Confirmatory Test)

Principle

The present immunoblot makes use of gene technology produced virus antigen. Four recombinant HCV antigens are used into the test strips:

- Core, Capsid antigen.
- NS-3, encoding the viral protease and helicase.
- NS-4, N-terminal part of NS.

NS4-1, internal part of NS-4; this antigen is fused to a foreign protein part. An isolated reaction to this protein could be caused by the foreign fusion part. The purified recombinant antigens are separated by molecular weight via polyacrylamide gel electrophoresis and subsequently transferred to a matrix. Caused by their gene technological origin, the recombinant antigens show values differing from their original molecular weight. Free binding sites on the matrix are saturated with neutral molecules. After incubation the strips with samples (serum, plasma) unbound antibodies are rinsed off. In a second incubation with serum containing antibodies conjugated with horse-radish peroxidase against human IgG and a subsequent enzymatic color reaction, specifically bound antibodies against virus antigens are detected [12].

3- Determination of β 2 Microglobulin in human serum

Principle

Is a quantitative test used on the Mini VIDAS instrument, for the measurement of β 2 M. The assay principle combines 2 steps enzyme immunoassay sandwich method with a final fluorescent detection (ELFA).

The β 2 M in the sample binds with specific monoclonal antibody coating the interior of the SPR. Unbound sample components are eliminated during the washing steps. The retained β 2 M is revealed by alkaline phosphatase-labeled polyclonal anti-human β 2 M antibody (sheep). Unbound conjugate is eliminated during the washing phase.

During the final detection step, the substrate (N-methyl umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl umbelliferone), the fluorescence of which is measured at 450 nm, the intensity of the fluorescence is proportional to the concentration of β 2 M present in the sample. The detailed procedure was carried out as has been suggested in the leaflet supplied with the test kit (biomerieux, France)[13].

Statistical analysis

The usual statistical methods have been used in order to assess and analyze our results and included:

Descriptive statistics: including

a. Mean (M).
b. Standard deviation (SD).
c. Statistical tables.

Inferential statistics: Data have been analyzed statistically using SPSS program version 10. Analysis of

quantitative data was done using t-test and ANOVA (analysis of variance). Acceptable level of significance was considered to be below 0.05[14].

Results and Discussion:

In fact, age at infection seemed to be the most influencing factor in prognosis, table (1) showed the distribution of hepatitis C patients according to age. It was found that the age of pre-treatment patients ranged between (20-65) years, with a mean age of 48.12 ± 16.1 while the age of post-treatment patients was found to be between (23-63) years, with the mean of 46.1 ± 18.1. Also it was found that more than half of patients have been located within third and fourth decade (i.e 30-49) year, with a percentage of 52.4% and 55.6 % for pre-treatment and post-treatment patients respectively as shown in table (1). These results coincided with [15,16] who found that the most common age group for hepatitis C was in fourth decade as well as [17] also reported that the most common age group was thirties for hepatitis c patients.

Moreover , regarding both groups, the males are more than females with the ratio of (3.2:1) among pre-treatment group and (2:1) among post-treatment group. The sex differences among both groups could be explained on the basis that males may have a greater chance to come in contact with risk factors of HCV than females, or alcohol intake being common in males, which may enhance the liver damage caused by HCV infection (1).Additionally [18,19] reported that males were represented significantly more frequent than females in the HCV antibody-positive group , besides [17, 20] found that CHC among males was more than females with a ratio of 1.7:1 and 2:1 respectively.

Table 1: Distribution of patients according to age

	pre-treatment group		post-treatment group	
Age groups (years)	Number	%	Number	%
20-29	7	11.1	5	9.3
30-39	15	23.8	13	24.1
40-49	18	28.6	17	31.5
50-59	12	19	10	18.5
60+	11	17.5	9	16.6
Total	63	100	54	100
Mean age (years)	48.12±16.1		46.1 ±18.1	

Table 2: Sex distribution of hepatitis C patients

	pre-treatment group		post-treatment group	
Sex	Number	%		
Male	48	76.2	36	66.7
Female	15	23.8	18	33.3
Total	63	100	54	100
M/F ratio	3.2:1		2:1	

Table (3) shows the concentration of β2 microglobulin before and after treatment with interferon among hepatitis C patients and the comparison between them. It has been found the concentration of β2 microglobulin was (3.425±0.943mg/L) among pre-treatment group and (1.860±0.723 mg/L) among post-treatment group with significant correlation (P=0.05). These results were in agreement with [20] who found that serum beta-2 microglobulin levels were significantly higher in pre-treatment group vs post-treatment group.

Beta2-microglobulin is a subunit of the class 1 major histocompaitibility complex found on the surface of all nucleated cells, including lymphocytes. Serum levels of β 2 M are produced during cellular turnover and its increases usually reflect an indirect state of generalized lymphoid activation [21]. Although raising blood levels of β- 2 M is found in patients with cancer and other serious diseases, as well as a rising β- 2 M blood level can be used to measure the progression of hepatitis [22]. In the present study, the level of serum β - 2 M is higher in pre-treatment group than pre-treatment

group. This is in agreement with the results of [8] they have noticed that the elevation of serum β - 2 M has been significantly associated with progression of chronicity of hepatitis and also they have observed that HCV–infection is strongly correlated with increased β - 2 M levels during the prognosis of disease .

Besides that , in the present study ,It has been found the concentration of β2 microglobulin was decrease after treatment from (3.425±0.943 mg/L) to (1.860±0.723mg/L) which was statistically significant (P=0.05) , as a result β2 microglobulin can be used as a supporting marker of responsiveness to treatment with interferon in hepatitis C patients . This study confirms the idea that beta2 microglobulin concentration is an indicator for monitoring the disease progression , which would lead to early initiation of interferon treatment and to monitor the effectiveness of the therapy.

Table 3 : Comparison between pre-treatment hepatitis C group and post-treatment group regarding beta 2 microglobulin

Marker	pre-treatment group (mean±SD) mg/l	post-treatment group (mean±SD) mg/l	P.value	significance
Beta 2 microglobulin	3.425±0.943	1.860±0.723	0.05	S

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دراسة مستوى تعبير جين beta 2 microglobulin في مرضى التهاب الكبد الفيروسي سي قبل وبعد العلاج بالانترفيرون

محمد عبد الدايم صالح*

*قسم علوم الحياة ،كلية العلوم، جامعة ديالى.

الخلاصة:

أجريت هذه الدراسة لتقييم مستوى تعبير جين beta2 microglobulin في مرضى التهاب الكبد الفايروسي سي قبل وبعد العلاج بعقار الانترفيرون .شملت الدراسة 117 مريض مصاب بالتهاب الكبد الفايروسي سي مؤلفين من 63 مريض غير معالج وبعمر يتراوح من 20-65 سنة وبمعدل عمري قدره 48.12±16.1 و54 مريض معالج وبعمر يتراوح من 23-63 سنة وبمعدل عمري قدره 46,1±18,1 وكذلك أوضحت الدراسة بان أكثر من نصف المرضى يقعون ضمن العقد الثالث والرابع من العمر (30-49 سنة) وبنسبة 52,4% للمجموعة غير المعالجة و55,6% للمجموعة المعالجة ومن جهة أخرى كانت نسبة الذكور أعلى من الإناث ولكلا المجموعتين 3.2:1 للمجموعة غير المعالجة و2:1 للمجموعة المعالجة ومن ناحية أخرى كان تركيز beta2 microglobulin بالنسبة للمجموعة غير المعالجة (3.425±0.943mg/L) و(1.860±0.723mg/L) بالنسبة للمجموعة المعالجة مع وجود فرق معنوي (P=0.05) وبالإضافة إلى ذلك بان نتائج الدراسة أوضحت بان تركيز beta2 microglobulin انخفض بعد العلاج من (3.425±0.943 mg/L)إلى (1.860±0.723mg/L) مع وجود فرق معنوي (P=0.05) وكنتيجة لذلك يمكن استخدام beta2 microglobulin كواسمة داعمة لمعرفة الاستجابة العلاجية لعقار الانترفيرون في مرضى التهاب الكبد الفايروسي سي وكمؤشر لمراقبة تتطور المرض