

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

*Mohammed A. Saleh\**

Received 26, June, 2010

Accepted 13, January, 2011

### 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  $48.12 \pm 16.1$  and 54 post-treated patients with age range was between 23-63 year with the mean of  $46.1 \pm 18.1$ . 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  $\beta 2$  microglobulin was ( $3.425 \pm 0.943$ mg/L) among pre-treatment group and ( $1.860 \pm 0.723$  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  $\beta 2$  microglobulin was decrease after treatment from ( $3.425 \pm 0.943$  mg/L) to ( $1.860 \pm 0.723$ mg/L) which was statistically significant ( $P=0.05$ ) , Thus  $\beta 2$  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].

$\beta 2$  - 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  $\beta 2$  microglobulin

\*Biology Department, College of Science , Diyala University.

protein is encoded by the B2M gene.  $\beta$ 2 microglobulin lies lateral to the  $\alpha$ 3 chain on the cell surface. Unlike  $\alpha$ 3,  $\beta$ 2 has no transmembrane region. Directly above  $\beta$ 2 (i.e. away from the cell) lies the  $\alpha$ 1 chain, which itself is lateral to the  $\alpha$ 2.  $\beta$ 2 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-2 microglobulin 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 possibly disrupts viral replication. There is evidence that IFN has a direct anti-viral action via induction of 2,5 A oligoadenylate synthetase and ribonuclease - 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 is 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  $\mu$ l) 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 pre-coated 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 $\beta$ 2 Microglobulin in human serum

### Principle

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

The  $\beta$  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  $\beta$  2 M is revealed by alkaline phosphatase-labeled polyclonal anti-human  $\beta$  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  $\beta$  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  $48.12 \pm 16.1$  while the age of post-treatment patients was found to be between (23-63) years, with the mean of  $46.1 \pm 18.1$ . 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**

Age groups (years)	pre-treatment group		post-treatment group	
	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**

Sex	pre-treatment group		post-treatment group	
	Number	%	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  $\beta 2$  microglobulin before and after treatment with interferon among hepatitis C patients and the comparison between them. It has been found the concentration of  $\beta 2$  microglobulin was ( $3.425 \pm 0.943 \text{mg/L}$ ) among pre-treatment group and ( $1.860 \pm 0.723 \text{mg/L}$ ) 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 histocompatibility complex found on the surface of all nucleated cells, including lymphocytes. Serum levels of  $\beta 2 \text{ M}$  are produced during cellular turnover and its increases usually reflect an indirect state of generalized lymphoid activation [21]. Although raising blood levels of  $\beta - 2 \text{ M}$  is found in patients with cancer and other serious diseases, as well as a rising  $\beta - 2 \text{ M}$  blood level can be used to measure the progression of hepatitis [22]. In the present study, the level of serum  $\beta - 2 \text{ M}$  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  $\beta$  - 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  $\beta$  - 2 M levels during the prognosis of disease .

Besides that , in the present study ,It has been found the concentration of  $\beta$ 2 microglobulin was decrease after treatment from (3.425 $\pm$ 0.943 mg/L) to (1.860 $\pm$ 0.723mg/L) which was statistically significant (P=0.05) , as a result  $\beta$ 2 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 $\pm$ SD) mg/l	post-treatment group (mean $\pm$ SD) mg/l	P.value	significance
Beta 2 microglobulin	3.425 $\pm$ 0.943	1.860 $\pm$ 0.723	0.05	S

### References:

- 1- Lauer, G.M. and Walker, B.D. 2001.Hepatitis C virus infection. *N Engl J Med.*; 345: 41-52.
- 2- Kittleson, D.J.; Chianese-Bullock, K.A.; Yao, Z.Q.; Bracial, T.J.; and Hahn, Y.S. 2000. Interaction between complement receptor gC1qR and hepatitis C virus core protein inhibits T-lymphocyte proliferation. *J.Clin. Invest.*; 106(10): 1239-1249.
- 3- Gong, G.Z.; Lai, L.Y.; Jiang, Y.F.; He, Y.; and Su, X.S. 2003. HCV replication in PBMC and its influence on interferon therapy . *World J. Gastroentrol.*; 9 (2): 291-294.
- 4- Weltman, M.D.; and Tally, N.J. 2003. Chronic hepatitis C infection: a review and update on treatment strategies .*ADF Health*; 4(1): 27-33.
- 5- Saab, S.; and Martin, P. 2000. Tests for acute and chronic viral hepatitis. *The practical peer-Reviewed J. for primary care phycicians*; 107(2): 123-130.
- 6- Girndt, M.; Sester, U.; Sester, M.; Deman, E. and Kohler, H. 2001.The Beta-2-microglobulin and interleukin - 10 promoter genotype determines clinical immune function in hemodialysis patients. *Kidney Int.*; 60: 2385 - 2391.
- 7- Urbani, S.;Boni, C.;Missale, G.; Cavallo, C. and Massari, M. 2007. Virus- specific CD8+ lymphocytes share the same effector- memory phenotype but exhibit functional differences in acute hepatitis B and C. *J.Virol.*; 76: 12423-12434.
- 8- Antonaci, S.; Piazzolla, G. and Napoli ,N. 2001. Relationship between T lymphocyte responsiveness (T helper 1/ T helper 2 type cytokine release) and Beta-2-microglobulin in chronic hepatitis B and C. *Micro.*; 106:203-209.
- 9- Bingham, F. 2004. How to monitor your blood work, in conjunction with DAIR laboratory Flow sheet and graph. *Hepatitis* ; 6:19-26.
- 10- Manns, M.P.; Mchutchion, J.G.; and Collins, S. C. 2001. Peg interferon alpha-2b plus ribavirin for intial treatment of chronic hepatitis C. a randomised trail. *Lancet*; 358: 958-965.

- 11- Countreras, M. and Barbara, J. 1989. Screening for hepatitis C antibodies. *The Lancet*; 2:505.
- 12- Gretch, D.R. 1997. Diagnostic test for hepatitis C. *Hepatology*; 26:43-47.
- 13- Winchester, J.F.; Salsberg, J.A. and Levin, N.W. 2004. "Beta-2 microglobulin in ESRD: an in-depth review.". *Adv. in renal replac. ther.*; 10 (4): 279-309.
- 14- Sorlie, D.E. 1995. Medical biostatistics and Epidemiology : Examination and board review. First ed. Appleton and Lange, Norwalk, Connecticut. P : 47-88.
- 15- Ghadhban, J.M. 1997. Prevalence of serological marker of hepatitis B virus ( HBs Ag ) and hepatitis C virus ( HCV) antibodies; among blood donors patients with chronic liver disease and certain risk groups. M.Sc. Thesis of Iraqi commission for medical specialization in medicine.
- 16- Zeuzem, S.; Fienman, V. and Rasenak, J. 2000 .Peg interferon alfa 2a in patients with chronic hepatitis C. *N. Engl. J. Med.*; 343: 1666-1671.
- 17- Coverdale, S.A.; Khan, M.H. and Byth, K. 2004. Effect of interferon treatment response on liver complication of chronic hepatitis C : 9 years follows up study. *Am. J. Gastroenterology*; 2 : 636.
- 18- Stapleton, J.T.; Klinzman, D.; Schmid, W.N.; Pfaller, M.A.; Wu, P.; Labrecque, D.R.; Han, J.; Phillips, M.J.P.; Woolson, R.; and Alden, B. 1999. Prospective comparison of whole- blood and plasma- Based hepatitis C virus- RNA detection system: Improved detection using whole blood as the source of viral RNA. *J.Clin. Microbiol.*; 73(3): 484-489.
- 19- Yoshida, H.; Arakawa, Y. and Sata, M. 2002. Interferon therapy prolonged life expectancy among chronic hepatitis C patients. *Gastroenterology*; 123: 483-487.
- 20- Luisa-García, B. ; Miguel - López, B.; Asunción- García-S.; and María, A. 2005. Variability in the expression of a  $\beta$ 2- microglobulin epitope on hepatocytes in chronic type C hepatitis on treatment with interferon. *Hepatology* ; 17(3), : 372-382,
- 21- Bardeguéz, A.D. ; Connor, E. and Stephens, R. 1999. Effect of human immunodeficiency virus infection on serum Beta 2 microglobulin level in pregnant women. *Obstet. and Gynecol.*; 94:537 -542.
- 22- Wells, K.R. and Odle, T.G. 2004. Effect of hepatitis on serum Beta 2 microglobulin level. *World J Gastroenterol.*; 94:547 -552.

## 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 الفيروسي سي قبل وبعد العلاج بعقار الانترفيرون. شملت الدراسة 117 مريض مصاب بالتهاب الكبد الفيروسي سي مؤلفين من 63 مريض غير معالج وبعمر يتراوح من 20-65 سنة وبمعدل عمري قدره 16.1±48.12 و 54 مريض معالج وبعمر يتراوح من 23-63 سنة وبمعدل عمري قدره 18.1±46.1 وكذلك أوضحت الدراسة بان أكثر من نصف المرضى يقعون ضمن العقد الثالث والرابع من العمر (30-49 سنة) وبنسبة 52,4% للمجموعة غير المعالجة و 55,6% للمجموعة المعالجة ومن جهة أخرى كانت نسبة الذكور أعلى من الإناث ولكلا المجموعتين 3.2:1 للمجموعة غير المعالجة و 2:1 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) وكننتيجة لذلك يمكن استخدام beta2 microglobulin كواسمة داعمة لمعرفة الاستجابة العلاجية لعقار الانترفيرون في مرضى التهاب الكبد الفيروسي سي وكمؤشر لمراقبة تطور المرض