TGF-B_1 IN PATIENTS WITH CHRONIC HBV INFECTION

Ali A. Ghweil[1], Usama A. Arafa. [2],Eman M Salah [3],Ali M Sayed [3]Ahmed N. Salem, Ashraf Khodeary[4],

[1]Tropical Medicine and Gastroenterology, South Valley University, Qena, [2]Internal Medicine, [3]Pathology, [4]Clinical Pathology, Sohag University, Sohag, Egypt.

ABSTRACT

Background: *The transforming growth factor-β_1 (TGF-β_1) is an important cytokine with anti-inflammatory properties may have a role in pathogenesis of liver fibrosis.* ***Objectives****: The main purpose of this study was to compare the serum levels of TGF-β_1 in a group of chronic HBV infected (CHB) patients as well as healthy individuals and to determine the correlation between the TGF-β_1 and stages of fibrosis in CHB patients.* ***Patients and methods:*** *A case control study using forty patients with CHB as well as forty healthy individuals were enrolled in the study. ELISA technique was applied to measure the serum level of TGF-β_1 in both patient and control groups. We used the data of the liver biopsy of CHB patients to make a correlation between TGF-β_1 and stages of fibrosis.* ***Results****: Our results revealed that the serum levels of TGF-β were significantly increased in CHB patients (1958.0±730.26pg/ml) in comparison with healthy controls (944.4±573.24 pg/ml) (P<0.0001). Serum levels of TGF-β_1 was significantly increased in F2-F3 (2600.0 ± 472.69 pg/ml) in comparison with F0-F1(1483.5 ± 478.54 pg/ml) (P < 0.0001).* ***Conclusion:*** *The study concluded that high serum levels of TGF-β may be a mechanism by which immune response against HBV is suppressed. The serum level of TGF-β_1 is a potential noninvasive maker for diagnosis of liver fibrosis in CHB patients.*

Keywords: TGF-β_1, Chronic HBV Infection, Stages of liver fibrosis.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection affects 350 million individuals worldwide. At least one million people chronically infected with HBV would die of chronic liver diseases each year (Lee WM., 2997). Thus, it is important to prevent the progression of early liver fibrosis to cirrhosis (Afdhal NH and Nunes D., 2004).

Although liver biopsy is the gold standard for the assessment of fibrosis, it has several disadvantages, such as poor patient compliance, sampling error, limited usefulness for dynamic surveillance, and poor intra- and inter-observation concordance (Little AF., 1996), (Lindor et al .,1996) and (Cadranel et al ., 2000) Considering these limitations, noninvasive histology predictors are urgently needed (Friedman SL.,2003).

Cytokines are small glycoproteins that are produced by immune cells and are involved in both inflammatory and anti-inflammatory reaction during diseases and heath (Arababadi et al., 2010).

Cytokines affect many functions in the liver, including amino acid, protein, lipid, mineral, and carbohydrate metabolism. In liver disease, cytokines are involved in the onset of intrahepatic immune responses, in liver regeneration, and in the fibrotic and cirrhotic transformation of the liver after chronic chemical injury or viral infection (Andus et al., 1991).

The TGF-β_1 is a famous member of anti-inflammatory cytokine family that is produced during hemostasis and tissue remodeling (Regateiro et al., 2011). Therefore, it is plausible to hypothesize that its up or down-regulation may lead to inappropriate immune responses against viral hepatitis. Because, chronic hepatitis B (CHB) patients are unable to completely eradicate HBV from their hepatocytes (Assar et al ., 2012) a probable mechanism has been proposed in which chronic up-regulation of TGF-β cause's

decreased degree of immune responses to the infected hepatocytes (Arababadi et al .,2010).

Therefore, we aimed to determine the serum levels of TGF-β_1 in the patients with CHB compared with healthy individuals and determine the correlation between the TGF-β_1 and stages of fibrosis in CHB patients.

PATIENTS AND METHODS

Forty CHB patients who underwent a liver biopsy in hospitals of Qena, and Sohag Faculties of Medicine, South Valley and Sohag Universities were included in this study. Also forty healthy subjects with matched age and sex were included in the study as a control group.

Chronic HBV infection was diagnosed based on positive surface antigen of HBV (HBsAg) for at least 6 months. Exclusion criteria included chronic liver disease due to other causes or co-infection with hepatitis C, clinically overt cirrhosis, previous or concomitant anti-HBV therapy, alcohol consumption.

Written informed consent was obtained from all patients and healthy subjects and the study was approved by the Local Ethics Committees in both faculties.

Liver histology and quantification of fibrosis:

Liver tissue was obtained by sono-guided percutaneous biopsy and stained with hematoxylin-eosin and Masson's trichrome. Fibrosis staging (F) and inflammatory activity (A) were decided according to the METAVIR system. Fibrosis staging was divided into F0-F4; F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = periportal fibrosis with few septa, F3 = septal fibrosis with many septa, and F4 = cirrhosis. The activity was assessed by integrating the severity and intensity of piecemeal (periportal) and lobular necrosis Inflammatory activity was divided into A0-A3; A0 = no histological necroinflammatory activity, A1 = minimal activity, A2 = moderate activity, A3 = severe activity (Khorramdelazad et al., 2012). According to the American Association for the Study of Liver Disease Practice Guidelines, we defined significant fibrosis as METAVIR fibrosis with a score ≥ 2 (F2, 3, 4) and severe liver fibrosis as METAVIR fibrosis with a score ≥ 3 (F3, 4) (Lebensztejn et al ., 2014).

Blood sampling and investigations:

Blood samples were collected in 3 tubes, the first one for complete blood count (CBC), the second for prothrombin time (PT) and the third containing no anticoagulant for the other investigations. The serum stored at -80°C for further test.

The laboratory investigations included aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, bilirubin , prothrombin time and concentration, complete blood count (CBC) and HBV-DNA-PCR for CHB group. HBsAg , HCV antibodies and TGF-β_1 were assayed for both the patients and control groups.

AST, ALT, bilirubin and albumin were determined by BECKMAN COULTER AU480 autoanalyzer system. PT was tested with Sysmex CA1500. CBC was assayed with Cell Dyn 3700 hematology analyzer, ABBOTT laboratories diagnostic division.

Enzyme-linked immunosorbent assay (ELISA) for the detection of HBsAg and HCV antibodies were performed by AXSYM system, ABBOTT laboratories.

TGF-β_1:

Measurement of serum TGF-β_1 was carried out on programmable Thermo ELISA reader (Finland) using KOMABIOTECH TGF-β ELISA kit (Korea) and according to the manufacturer's guidelines. Data were only used when the inter and intra-assays produced the scores of CV < 14% and CV < 3%, respectively.

HBV-DNA Extraction and polymerase chain reaction (PCR):

Viral DNA was purified from 200 µL of plasma from HBsAg positive patients using a QIAamp MiniElute Virus spin kit (QIAGEN-Germany) for DNA purification performed by QIAcube fully automated extractor (Germany) according to the manufacturer's guidelines. HBV-

DNA quantification was also done using a QuantiFast Pathogen PCR Kit (QIAGEN-Germany) following the manufacturer's instructions. The viral load considered low viremia when the HBV- DNA was less than 100,0000 copies /ml , moderate viremia form 100,000-1000,000 copies/ml and high viremia over 1000,000 copies/ml.

Statistical analysis :

The parametric statistical analysis were performed using the SPSS software version 18 and the P value of less than 0.05 considered as significant.

RESULTS

The mean age of the 40 CHB patients (28 males, 12 females) was 27.6 ± 8.3 years. The mean age of the 40 control subjects (27 male, 13 females) was 26.6 ± 7.2 years. The mean levels of platelet count and albumin were significantly lower in patients with F2-F3 fibrosis than in those with F0-F1 fibrosis, while the prothrombin time is significantly higher in patients with F2-F3 fibrosis than in those with F0-F1 fibrosis. The main laboratory data of the patients are summarized in table (I).

Results of this study showed that serum levels of TGF-β_1 was significantly increased in CHB patients (1958.0±730.26pg/ml) in comparison with the healthy control subjects (944.4±573.24 pg/ml) ($P < 0.0001$) as shown in figure (1). Serum levels of TGF-β_1 was significantly increased in F2-F3 (2600.0 ± 472.69 pg/ml) in comparison with F0-F1(1483.5 ± 478.54 pg/ml) ($P < 0.0001$) as shown in figure (2). There was significant positive correlation between TGF-β_1 and stages of fibrosis ($P < 0.0001$) as shown in figure (3). There was no significant correlation between the serum level of TGF-β_1 and the HBV-DNA viral load (copy number) ($P > 0.05$) (Figure 4).

Table (I): The laboratory data in F0 & F1 and F2 & F3 groups.

Parameter	F0 & F1 (N=23) Mean± SD	F2 & F3 (N=17) Mean± SD	All patient (N=40) Mean± SD	P value
Bilirubin(mg/dl)	**0.57±0 .273**	**1.07± .408**	**0.78±.415**	**<0.0001**
AST(IU/L)	**22.3± 11.53**	**22.4± 14.05**	**22.3±12.49**	**0.967**
ALT(IU/L)	**22.2 ±8.60**	**21.8± 10.48**	**22.0±9.32**	**0.897**
Albumin (g/dl)	**3.94± .388**	**3.20± .452**	**3.62±.554**	**<0.0001**
PT(second)	**12.1± 0.84**	**15.7 ± 0.92**	**13.9±1.2**	**<0.0001**
WBCs ($x10^9$/L)	**5658.7± 1271.45**	**5182.3± 2116.08**	**5456.2±1675.06**	**0.381**
Hb (g/dl)	**13.06± 1.517**	**12.76± 2.107**	**12.93±1.773**	**0.603**
Platelet ($x10^9$/L)	**203.6± 39.45**	**112.4± 17.40**	**1958.0±730.26**	**<0.0001**
TGF-β_1 (pg/ml)	**1483.5± 478.54**	**2600.0± 472.69**	**1958.0±730.26**	**<0.0001**

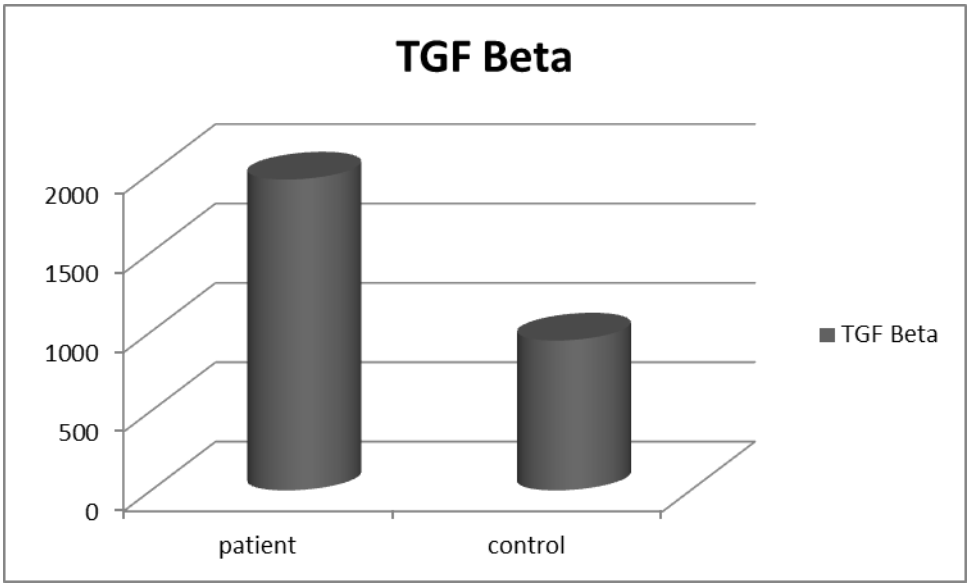

Figure (1): Serum TGF-β_1 in patients and control groups (P < 0.0001).

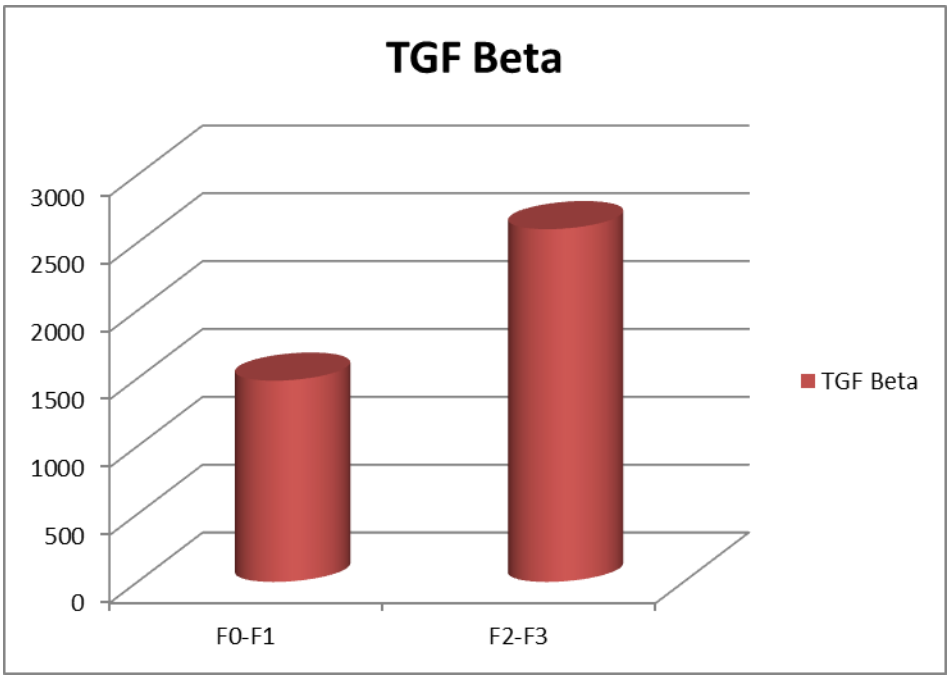

Figure (2): Serum TGF-β_1 in F0-1 and F2-3 (P < 0.000).

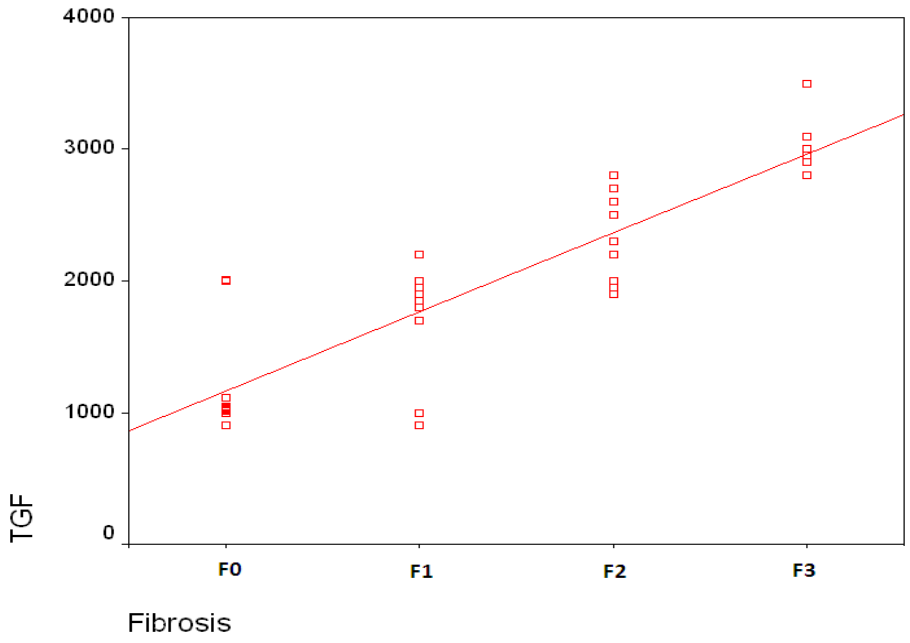

Figure (3): Correlation between serum TGF-β_1 and stages of fibrosis(P < 0.000, r o.87)

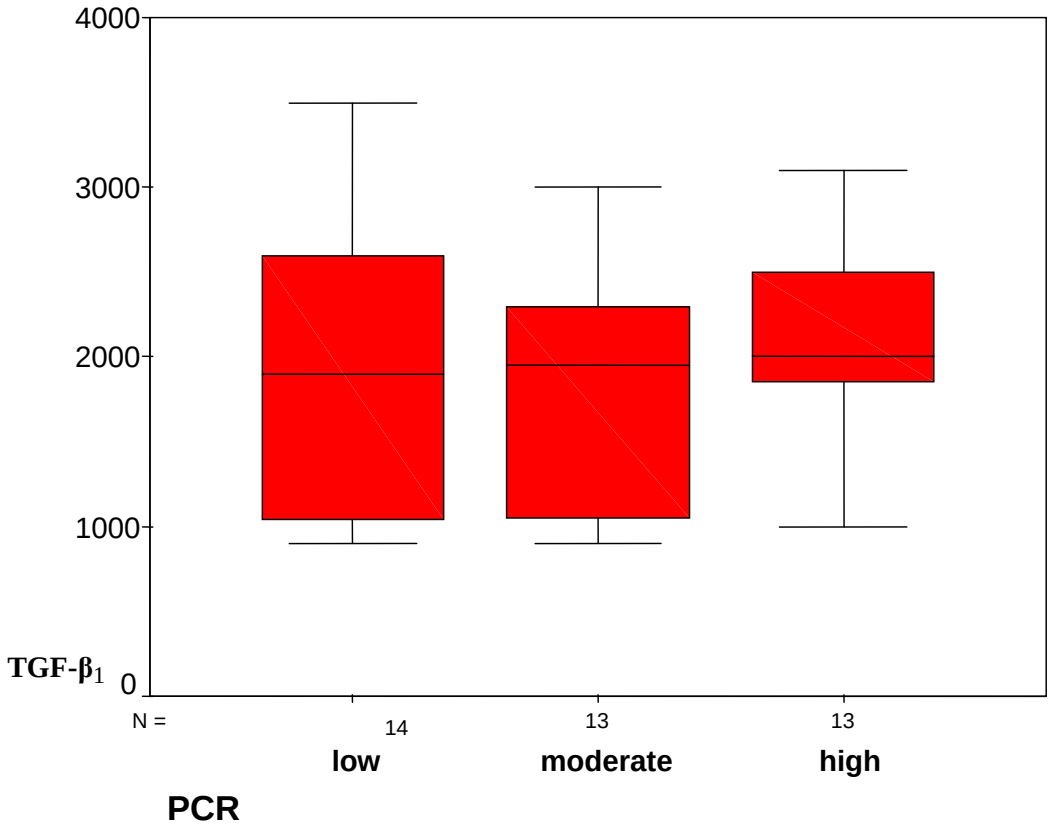

Figure (4): Correlation between the serum TGF-β_1 and viral load (p< 0.67)

DISSCUSION

The cytokines network plays important role during immune response against intracellular infections including viral infection (Arababadi MK et al., 2012). TGF-β_1, as an anti-inflammatory cytokine, plays key roles in the regulation and suppression of immune responses (Regateiro FS et al ., 2011).. Therefore, any significant alteration in the expression of TGF-β_1 may lead to inappropriate immune responses against viral infections. TGF-β1 is an important cytokine in the patho-physiology of liver fibrosis, stimulating the production of extracellular matrix. In hepatic fibrosis, a marked increase is seen in the hepatic extracellular matrix proteins, including collagens, glycoproteins, and lycosaminoglycans. In both experimental models of hepatic fibrosis and in patients with liver cirrhosis, increased expression of type I collagen genes is seen (Panduro et al., 1988). The TGF-β_1 also stimulate type I collagen gene expression in primary cultures of hepatocytes, Ito cells, and fibroblasts (Czaja et al., 1989).

Transforming growth factor beta1 or TGF-β_1 is a polypeptide member of the transforming growth factor beta superfamily of cytokines. It is a secreted protein that performs many cellular functions, including the control of cell growth, cell proliferation, cell differentiation and apoptosis (Ghadami et al., 2000), (Vaughn et al., 2000).

The results of this study showed that the serum level of TGF-β_1 is significantly increased in CHB patients in agreement with Khorramdelazad et al. . Increased serum levels of TGF-β_1 in CHB patients also were reported by Lebensztejn et al. (Lebenszejn et al ., 2004) and Akpolat et al. (Akpolat et al ., 2005).

Our study showed significant positive correlation between the serum level of TGF-β and different stages of liver fibrosis and this match with results obtained by Gue et al (Gue et al., 2008) who suggested close relation between the level of TGF- β1 and the different liver fibrosis grades due to chronic hepatitis B.

Our study showed there is no significant correlation between the serum level of TGF-β_1 and the HBV-DNA viral load in agreement with Khorramdelazad et al. (Khorramdelazad et al., 2012).

In agreement with our study Castilla et al (Castilla et al., 1991) have shown that the level of TGF β_1 mRNA in liver biopsy specimens correlated positively with hepatic fibrosis in a large group of patients with chronic viral hepatitis, suggesting that TGF- β1 may play a role in the pathogenesis of hepatic fibrosis.

So we can conclude that The serum level of TGF- β_1 is a potential noninvasive maker for diagnosis of liver fibrosis in CHB patients.

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معامل النمو التحولي بيتا 1 ($TGF\text{-}\beta_1$) فى المرضى المصابين بعدوى فيروس الإلتهاب الكبدي (ب) المزمنة

د. على عبدالرحمن غويل - د. أسامه عرفه – د. أحمد نورالدين سالم – د. إيمان محمد صلاح الدين– د. على محمد سيد – د.أشرف خضيرى

مقدمة : يعتبر معامل النمو التحولي بيتا 1 من المركبات الخلوية ذات الخواص المضادة للإلتهاب وقد يكون له دور فى تكوين تليف الكبد.

أهداف البحث : الهدف الرئيسي لهذا البحث هو دراسة معدل معامل النمو التحولي بيتا 1 فى الدم فى مجموعة من المرضى المصابين بعدوى فيروس الإلتهاب الكبدي (ب) المزمنة بالمقارنة بمعدله فى مجموعة ضابطة من الأشخاص الأصحاء ودراسة مدى العلاقة بين معامل النمو التحولي بيتا 1 و مراحل التليف فى المرضى المصابين بعدوى فيروس الإلتهاب الكبدي (ب) المزمنة.

طرق البحث : اشتملت هذه الدراسة على عدد 40 مريضا مصابين بعدوى فيروس الإلتهاب الكبدي (ب) المزمنة بالإضافة إلى 40 فردا من الأصحاء . و تم استخدام طريقة إليزا (ELISA) لقياس معدل معامل النمو التحولي بيتا 1 فى الدم فى المجموعتين . كما تم استخدام معلومات العينات الكبدية للمرضى لعمل مقارنة بين معامل النمو التحولي بيتا 1 و مراحل التليف.

النتائج : خلصت الدراسة إلى أن هناك زيادة ذات دلالة إحصائية فى معدل معامل النمو التحولي بيتا 1 فى المرضى المصابين بعدوى فيروس الإلتهاب الكبدي (ب) المزمنة بالمقارنة بمعدله فى المجموعة الضابطة. كما وجد أيضا زيادة ذات دلالة إحصائية فى معدل معامل النمو التحولي بيتا 1 فى مرحلتي التليف (F3 - F2) بالمقارنة بالمرحلتين (F1 - F0).

الخلاصة : إن المعدل المرتفع لمعامل النمو التحولي بيتا 1 ربما يلعب دورا فى تثبيط الإستجابة المناعية فى المرضى المصابين بعدوى فيروس الإلتهاب الكبدي (ب) المزمنة. ويمكن أن يستخدم كدالة سهلة لتشخيص مرحلة تليف الكبد فى المرضى المصابين بعدوى فيروس الإلتهاب الكبدي (ب) المزمنة.