Cloning, expression and identification in immunohistochemistry of humanized single-chain Fv antibody against hepatitis C virus core protein

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Objective: To clone and express humanized single chain Fv antibody against hepatitis C virus core protein and identify its application in immunohistochemistry.

Methods: The phage antibody library was panned by HCV core protein, which was coated in microtiter plate. After five rounds of biopanning, 60 phage clones were identified specific to HCV core protein. The selected scFv was subcloned into the vector pCANTAB5E for expression as E-tagged soluble scFv. The liver tissue sections from normal person and patients with chronic hepatitis C were immunostained.

Results: ELISA and immunohistochemistry study demonstrated that the human single chain Fv antibody against hepatitis C virus core protein has a specific binding character with paraffin-embedded tissues from patients with chronic hepatitis C, but did not react with liver tissues from healthy persons.

Conclusion: Humanized single chain Fv antibody to HCV core protein has been identified by means of the phage display technology.

HCV genotype distribution in chronic hepatitis C patients in a tertiary care hospital of Rawalpindi, Pakistan

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Background/Aims: Genotypes 1-6 have a worldwide distribution. Types 1a and 1b are predominant in Northern Europe and North America, and in Southern and Eastern Europe and Japan, respectively. Type 3 in south Asia. Genotype 4 in Egypt, genotype 5 in Central and South America and genotype 6 is common in China, Japan and South East Asia. In Pakistan 3a is the commonest genotype. As reported by different authors HCV genotype 3a is associated with the most favorable outcome regarding ETR and SVR after 24 weeks conventional Interferon and Ribavirin therapy. The aim of this study is to find out HCV Genotypes in newly diagnosed chronic hepatitis C patients.

Methods: This observational study was conducted in chronic hepatitis C patients treated with conventional interferon 3 MIU thrice weekly and ribavirin 400 mg bid for 24 weeks. All patients had raised ALT levels for last 06 months, had positive PCR for HCV RNA by real time method and liver biopsy was done during year 2006-2007. Genotyping was done for Roche Genotyping Kit. Data was analyzed by using SPSS 13.0

Results: Out of 164 patients, 85.9% (n=141) were genotype 3a.
PP-130  Virological response in relation to biochemical changes in chronic hepatitis C patients in a tertiary care hospital in Pakistan
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Background: Alanine aminotransferase is an enzyme produced in hepatocytes and its raised levels in serum indicate hepatocyte damage. Furthermore, the number of patients with sustained viral response (SVR) and with sustained ALT normalization in non-SVR patients was also significantly higher in the combination therapy versus monotherapy group.

Objectives: To study the effect of initial ALT levels on predicting sustained virological response in HCV infected patients getting conventional Interferon alpha 2b and Ribavirin combination therapy in the Gastroenterology and Hepatology division of Holy Family Hospital under the National program for prevention and control of hepatitis in Pakistan.

Methodology: Retrospective observational study on the records of patients diagnosed as HCV positive coming for conventional Interferon alpha 2b and Ribavirin combination therapy in the Gastroenterology and Hepatology division of Holy Family Hospital. The aim is to be noted that pathology lab at Holy Family Hospital takes a value less than or equal to 45 U/L as normal.

Results: (n=89): Group I: ALT ≤ 45
- n=25; SVR achieved 80.0%, n=20; Relapser 20.0%, n=05
Group II: ALT > 45
- n=33; SVR achieved 81.8%, n=27; Relapser 18.8%, n=06
Group III: ALT > 90
- n=31; SVR achieved 64.5%, n=20; Relapser 35.5%, n=11

Conclusion: It was concluded that there is significant PCR negativity at 24 weeks in patients with ALT levels up to 90 U/L as compared to the patients with ALT levels more than 90 U/L.

PP-131  Biochemical Response with interferon alpha 2b and ribavirin combination therapy in chronic hepatitis C patients
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Background/Aim: Alanine aminotransferase is an enzyme produced in hepatocytes and its raised levels in serum indicate hepatocyte damage. Conventional Interferon being an immune modulator and Ribavirin being a nucleoside analogue antiviral drug is believed to suppress hepatocyte damage and inflammation and so decrease the levels of ALT in serum of patients with high ALT levels undergoing combination therapy. The aim is to study the changes in ALT levels in HCV infected patients getting conventional Interferon alpha 2b and Ribavirin combination therapy

Methodology: Retrospective observational study on the records of patients diagnosed as HCV positive coming for conventional Interferon and Ribavirin therapy to the Gastroenterology and Hepatology division of Holy Family Hospital under the National program for prevention and control of hepatitis in Pakistan. It is to be noted that pathology lab at Holy Family Hospital takes a value less than or equal to 45 U/L as normal.

Results: (n=663): At the start of combination therapy the mean ALT levels were 80.20 with std. deviation of 66.176 and after 06 months of therapy the mean ALT levels were 41.47 with std. deviation of 42.194. Furthermore, 461 patients had ALT levels more than 45 before starting combination therapy and out of these 461 patients 69.6%, n= 321 had normalization of ALT levels (>45 IU/L).

Conclusion: It was concluded that use of Conventional Interferon alpha 2b and Ribavirin combination therapy in patients with chronic Hepatitis C, ALT levels show trend towards Normalization.

PP-132  Thrombocytopenia in chronic hepatitis C patients treated with conventional interferon and ribavirin in a tertiary care hospital of Rawalpindi, Pakistan
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Background: Thrombocytopenia is one of the major adverse effects of IFN-α and ribavirin often leading to dose reduction or treatment discontinuation. Recent data demonstrated that 4-6% of patients underwent dose modification secondary to thrombocytopenia. Reduction of the dose is recommended at 50,000 cell count/cmm and discontinuation, if the platelet count drops below 30,000 cell/cmm. It is now a known fact that dose reduction or interruption of therapy leads to lesser SVR.

Methods: This observational study was conducted in chronic hepatitis C patients treated with conventional interferon 3 MIU thrice weekly and ribavirin 400 mg bid for 24 weeks. All patients had raised ALT levels for last 06 months, had positive PCR for HCV RNA by real time method and liver biopsy was done. Platelet counts along with hemoglobin concentration and TLC were monitored in all patients on monthly basis. Data was analyzed by using SPSS 13.0

Results: Out of 422 patients 15% (n=63) developed thrombocytopenia at 12 weeks whereas out of the remaining 359 patients another 6% (n=22) developed thrombocytopenia at 24 weeks of therapy. 70 Patients had platelet count in the range of 100-150,000 cells/cmm. 11 patients had platelet count less than 100,000 cells/cmm and 02 patients had platelet count less than 50,000 cells/cmm.

Conclusion: We conclude that thrombocytopenia is present in a significant number of patients receiving conventional interferon and ribavirin therapy (15% at week 12 and 6% at week 24). 02 patients needed discontinuation of therapy.

PP-133  Correlation of alanine aminotransferase (ALT) levels with necroinflammatory score and stage of fibrosis on liver biopsy in chronic hepatitis C patients
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Background/Aims: Hepatitis C is the most common cause of Chronic Liver Disease in Pakistan with HCV prevalence of 4%. Liver Biopsy is done for suitability of treatment based on necroinflammatory score (grade) and stage of fibrosis. The aim of this study is to evaluate the staging and grading of histological changes on liver biopsy in Chronic Hepatitis C patients and its correlation with serum ALT levels.

Methods: Retrospective descriptive study on the patient records at Gastroenterology and Hepatology division of Holy Family Hospital including patients of Chronic Hepatitis C undergoing first liver biopsy with no prior treatment and excluding those with other indications for biopsy. Data was analyzed using SPSS 13.