INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 10, No. 4, p. 354-362, 2017

RESEARCH PAPER OPEN ACCESS

Factors linked to sustained virological response rates during antiviral therapy in chronic hepatitis-C patients from slums of Islamabad

Asad Zia[1], Muhammad Ali[1], Sohail Akhtar[3], Muhammad Zia[1], Abida Raza*[2]

[1]*Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan*

[2]*National Institute of Lasers & Optronics (NILOP), Islamabad, Pakistan*

[3]*Department of Statistics, University of Malakand, Pakistan*

Key words: Hepatitis C virus, IL 28B polymorphism, Ribavirin, Interferon, Sustain virological response rate

http://dx.doi.org/10.12692/ijb/10.4.354-362 Article published on April 30, 2017

Abstract

Human genetic and viral factors are the key factors correlated with treatment response and spontaneous clearness of HCV infections. In the current study, we investigate the possible correlation of HCV-3a genotype, viral load, host age, gender and IL28B (rs12979860) polymorphism with interferon plus ribavirin treatment. Sixty seven chronic HCV patients were selected for the current study to assess the dependence of treatment outcomes in patients undergoing combination therapy (IFN plus RBV). Patients with age ≥16 years, both gender males and females, having no co-infection with HBV or HIV, no liver complications e.g. (cirrhosis, fibrosis, and hepatocellular carcinoma) were included in the study. Twenty HCV infected patients were excluded from the study who did not qualify the criteria. Of the remaining sixty seven, 37 (55.22%) were female and 30 (44.77%) were male. For HCV genotyping, from the serum viral RNA was extracted and for host genetic factor IL28B (rs12979860) polymorphism was assessed, for this purpose, whole blood was used for extraction of genomic DNA, PCR amplification of 694 bp of IL28B (rs12979860) was carried out followed by IL28B typing by restriction fragment length polymorphism. We found dominated HCV genotype (HCV-3a) 62.68% (42/67) in slums of Islamabad followed by HCV-1a 26.86% (18/67) and 10.44% (7/67) were infected with untypable. For IL28B we found three types of polymorphism in rs12979860, the most prevalent polymorphism among the present study patricians is CC (81.57%) followed by CT (55.55%) while CT (9%) were rarely found. The SVR was significantly higher in patients with CC polymorphism at IL28B (rs12979860) ($p = 0.000$)and is not effected by other host factors such as gender ($p= 0.035$), age ($p= 0.352$).

* **Corresponding Author:** Abida Raza ✉ abida_rao@yahoo.com

Introduction

One of the main causes of liver complication and end stage liver diseases is hepatitis C virus (HCV). About 85% HCV infected individuals develop chronic disease and only 15% of individuals show spontaneous clearance.

The worldwide HCV prevalence approximately is 3% with about 200 million people infected globally (Messina *et al.* 2015). Pakistan is a developing region with the prevalence rate as high as 7%, placing it as the second most affected regions after Egypt which has prevalence rate of 14.7% (Aslam. 2016; El-Zanaty and Way. 2009; Frank *et al.* 2000; Shepard *et al.* 2005). HCV is becoming a main health issue in Pakistani population due to increase in poverty and lack of hygienic life style (Akbar *et al.* 2009). Furthermore, the country is at risk of increasing the HCV prevalence because of refugees and Internally Displaced People (IDP's), and resource limitations. Besides, the aforementioned factors deadly natural calamities like earthquakes and floods in the recent years have created a thriving environment for the spread of infections including HCV.

With limited resources, Pakistan faces numerous health challenges with HCV being serious because of the continuous increase in its prevalence. Henceforth, any study undertaken on the HCV prevalence and treatment is of significant importance in the current scenario (Khan and Qazi. 2013; Shinwari *et al.* 2014).

Some recently developed therapies have been introduced, combination therapy of RBV plus PEG-IFN stays a popular choice for decades. Nevertheless, some patients may experience some side effects and may not achieve sustained virological response. Moreover it is costly which makes it unaffordable to a large chunk of population suffering from the infection. Therefore it is imperative to underline the important factors which undermine or enhance its effectiveness (Aziz *et al.*, 2011; Fried. 2002; McHutchison *et al.*, 2009). Numerous factors controls the variation in outcome of treatment which can be host or pathogen related.

There are various host factors responsible for variability in treatment outcomes. Studies have shown a strong correlation among virus clearance, viral and host genetic factors. Moreover, natural clearance of virus is associated with strong immune system (Cooper *et al.* 1999; Rehermann and Nascimbeni. 2005).

Detailed Genome-wide association studies (GWAS) have demonstrated key single nucleotide polymorphisms (SNPs) in IL28B gene that have high correlation with viral clearance and treatment responses (18-21). Moreover, the validation of this correlation is globally done by studies reporting from different ethnic backgrounds (Mangia *et al.* 2010).

In numerous studies the altered behavior of variants of IL28B gene has been reported in treatment course and may be beneficial in terms of positive treatment out comes of combine PEG-IFN plus RBV (Ge *et al.* 2009; Moghaddam *et al.* 2011; Tanaka *et al.* 2009)

This study assessed the predictive power of rs12979860 IL-28B variants on the response to RBV plus IFN therapy in a group of patients from slums of Islamabad, Pakistan infected with chronic hepatitis C (CHC) genotype 3a without liver complications, in relation to other predictors of response.

In the present study we have reported the high dependence of RBV plus IFN response on IL28B polymorphism, and independence of age, gender, and HCV genotypes.

Materials and methods

Subjects

Department of Biotechnology QAU, Islamabad hosted the current study from February 2015 to January 2016. The present study was approved by departmental ethical committee, and written consent was obtained from patient.

Sixty seven chronically infected HCV patients that have baseline viral load higher and lower (than $\geq 5\times10^5$ IU/mL) from slums Islamabad were included.

For inclusion, patients had to complete 6 months combine therapy of IFN plus RBV, aged ≥16 years, both gender, and negative for coinfection of HIV and hepatitis B virus (HBV). Combined therapy of IFN-2a plus RBV 400 mg once daily was administrated.

Ethylenediaminetetra acetic acid (EDTA) - containing tubes were used to collect blood from the patients and stored at -80°C. All the participants were administrated with combined IFN-2a plus RBV for 24 weeks for chronic HCV infected patients.

The viral loads were checked at the third and sixth month of treatment as well as six months after the completion of treatment. Undetectable HCV RNA by real-time polymerase chain reaction (PCR) (≤20 IU/mL) at the completion of treatment was regarded as SVR.

Study design

Patients included in the study were evaluated whether, they show SVR or they are resistant to the prescribed treatment. The aim of this study was to assess the effect of rs12979860 genotypes on combine IFN plus RBV therapy outcomes. SVR is defined as Undetectable HCV RNA after six months of completion of therapy. At the 12 week of treatment the patients whose HCV RNA decline less than 2 log were non-responder.

All those patients that are HCV RNA positive during treatment follow up period and whom had previously cleared HCV RNA at the end treatment are defined as relapse. HCV RNA was extracted from 100 µl serum by RNA isolation kit (INSTANT, AJ Roboscreen, GmbH Germany). Genotyping were performed described somewhere else (Idrees. 2008; Ohno *et al.* 1997).

Polymorphism of the IL28B gene (rs12979860) was performed using methods (Aziz *et al.* 2015a; Moreira *et al.* 2012). Whole blood was used for the extraction of genomic DNA. IL28B gene rs12979860 (694bp region) was amplified using previously reported primer sets (Aziz *et al.* 2015b). Restriction digestion was performed by using *Hpy*166II and DNA fragments were detected on 2% agarose gel as shown in Fig. 1.

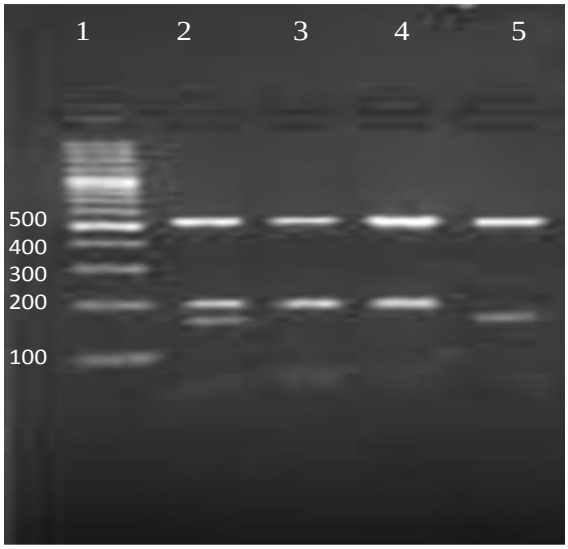

Fig. 1. RFLP of the amplified product of rs12979860: lane 1, 100-bp DNA ladder; lane 2, the electrophoresis pattern of genotype CT digested with Hpy166II enzyme; lanes 3 and 4, restricted PCR product of genotype CC; lane 5 genotype.

Statistical analysis

Continuous data are presented as mean and standard deviation (SD) values while categorical data are shown as frequencies and percentages. Comparison between the categorical variables was performed through Chi-square test.

Multivariable logistic regression models are used to investigate the potential risk factors which forecast the therapeutic outcome. SPSS software version 20.0 (SPSS, Chicago, IL, USA), was used to analyzed all data by considering, statistically significant P-value < 0.05.

Results and discussion

Sampling and baseline characteristics of patients and virus

Sixty seven patients were primarily, included in the current study to evaluate the dependence of outcomes of combined IFN plus RBV on various hosts such as IL 28B, age, gender and virus factors as baseline viral load and genotype.

Twenty five patients that did not the follow the inclusion criteria were excluded. Baseline, characteristics of 67 patients included in the present study (Table 1). Fig. 2 shows the electrophoresis pattern of IL28B (rs 12979860).

Table 1. Baseline characteristics of patients and virus with treatment induced HCV RNA clearance or HCV RNA persistence.

Baseline characteristic		Individuals with spontaneous HCV RNA clearance (N=42, 62.68%)	Individuals with HCV RNA persistence (N=25, 37.31%)	Total Patients (N=67)	P- value
SNPs	CC	31 (73.80%)	7(28%)	38	p=0.000
	SNPs CT	10 (23.80%)	8 (32%)	18	
	SNPs TT	1 (2.38%)	10 (40%)	11	
Gender	Male	20 (66.66%)	10 (33.33%)	30	p=0.035
	Female	22 (59.45%)	15 (40.35%)	37	
Age	Age <31	10 (52.26%)	9 (47.36%)	19	p=0.352
	31-50	18 (60%)	12 (40 %)	30	
	>50	14 (77.77%)	4 (22.22%)	18	
Genotypes	1a	12 (28.57%)	6 (24%)	18	p=0.982
	3a	26 (61.90%)	16 (64%)	32	
	Untypable	4 (9.52%)	3 (12%)	7	
Baseline viral load	≥500000	31(64.58%)	17 (35.41%)	48	p=0.062

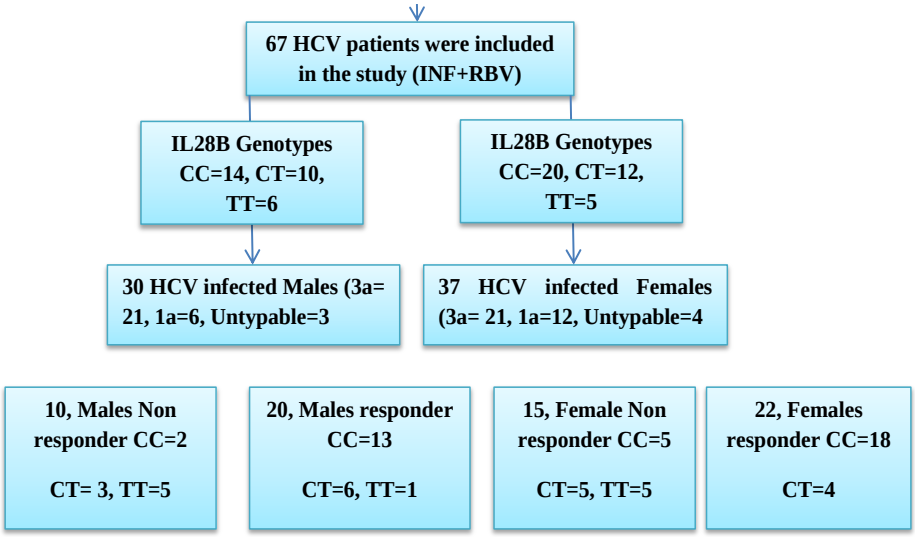

Fig. 2. Study diagram shows distribution of IL28B (rs12979860) and response rate to treatment.

Indicators of a sustained virological response

In all, 92 patients with chronic HCV infection who initially agreed to fulfill the study protocol were selected and screen. Twenty patients who did not comply with our designed protocol were excluded from the study. The remaining 67 patients who followed the protocol and completed the entire antiviral therapy course including 30 males and 37 females infected with HCV 3a, 1a and untaypable genotypes were including the study as shown in Fig. 2. In the present study different host and viral factors were compared that could be correlated in terms of response to antiviral therapy. Initial analyses revealed a non-significant dependence in SVR between males and females (66.66% vs. 59.45%; p = 0.035) and between different age groups (p = 0.352).

Baseline viral load low (<5×10^5 IU/mL) and high (>5×10^5 IU/mL) were found in significant (68.5% vs. 68.8%; p = 0.062). However, the results of SVR are highly correlated with polymorphism in IL28B gene (p = 0.000).

Table 2 demonstrates the multivariable logistic regression models analysis. It shows that there is no dependence in the SVR between female and male (odds ratio (OR) 0.14, 95% confidence interval (CI) 0.02–0.87; p = 0.55), or between those with viral loads >5× 10^5 IU/mL and <5× 10^5 IU/mL (OR 5.21, 95% CI 0.92–29.61; p = 0.062), no dependence was observed between different age groups (OR 1.02, 95% CI 0.98–1.07; p = 0.352), marital status was found non-dependent (OR 1.66, 95% CI 0.07–38.32; p = 0.751),

non-dependence in SVR between different viral genotypes was observed (OR 0.99, 95% CI 0.38–2.59; p = 0.982). Higher significant dependence in SVR between patients with different IL28B genotypes was observed (OR 0.99, 95% CI 0.03–0.3; p = 0.000). For the factors that contribute significantly to predict the therapy outcome multivariate logistic regression was done. Polymorphism at IL28B (rs12979860) were significantly correlated with SVR. The most significant genotype of rs12979860 is that CC has about two fold higher SVR as compare to CT and TT genotypes (OR 5.6, 95% CI 2.2–10.0; p = 0.000).

Table 2. Fitted parameter estimates using stepwise logistic regression. The Chi–square (χ^2) statistic was used to check the association between variables and factors.

	Coefficient	S.E Coefficient	Z-value	P-value	Odd ratio	95%CI	
Constant	5.4059	4.2382	1.280	0.202		Lower	Upper
Polymorphism	-2.4109	0.6155	-3.920	0.000	0.09	0.03	0.3
Gender	-1.9810	0.9419	-2.100	0.035	0.14	0.02	0.87
Age	0.0219	0.0235	0.930	0.352	1.02	0.98	1.07
Baseline viral load (IU/mL, %)	1.6514	0.8862	1.860	0.062	5.21	0.92	29.61
Genotypes	-0.0114	0.4918	-0.020	0.982	0.99	0.38	2.59

Discussion

Interferon plus ribavirin is the standard therapy in patients infected with HCV 3a genotype for 24 weeks (Dalgard *et al.* 2004). However, the studies report that the treatment outcome is not always uniform in all HCV infected patients. Various host and viral factors such as gender, age, ethnicity, host genome, genotype, viral load and co-infections collectively influence the treatment response (Ali *et al.* 2016; Kau *et al.* 2008; Wohnsland *et al.* 2007). In this study, we investigated the viral and host factors influencing the combine treatment outcome of IFN plus ribavirin in chronic HCV 3a infected patients. The study reports that, among host factors, only IL28B polymorphism (rs12979860) was significantly correlated with SVR to combine IFN plus RBV treatment. Other factors such HCV genotypes and host age, gender were in significant with treatment response. Furthermore, genotype 3a the most dominant HCV genotype (62.68%) in the slums population of the Islamabad followed by genotype 1a (26.86%) while, 10.44% were untypable. Idrees and Riazuddin preveviously reported that patients with CC genotype responded efficiently to IFN treatment regimen as compare to CT and TT genotypes of IL28B (rs12979860) (Asselah *et al.* 2011; Asselah *et al.* 2012; Idrees and Riazuddin. 2008). Our findings are in harmony with Khairy and colleagues and others, where in the most responsive genotype is CC that is highly correlated with high SVR in comparison to CT and TT genotype respectively in patients infected with genotype 4 (Asselah*et al.* 2012; Cieśla *et al.* 2012; De Nicola *et al.* 2012; Khairy *et al.* 2013; McCarthy *et al.* 2010; Rauch *et al.* 2010). A higher SVR has been reported for genotype 1 infected patients with rs12979860 CC polymorphism at IL28B treated with combine IFN plus RBV (Shaala *et al.* 2015). In present study, we reported that gender is not significantly correlated with responsiveness to combine IFN plus RBV treatment. Aziz and co-workers also reported no significant difference in SVR between females and males (Aziz *et al.* 2015b) . The prime finding of our study was that IL28B polymorphism rs12979860 was highly correlated with the positive treatment outcome of the combined IFN plus ribavirin in chronic HCV patients of the slums. According to the different groups that published the results of (GWAS) in 2009, IL28B polymorphism has strong effect on treatment induced clearance in chronic HCV infected patients (Ge *et al.* 2009; Rauch *et al.* 2010; Suppiah *et al.* 2009; Tanaka *et al.* 2009). However, Asahina and colleagues reported IL28B polymorphism and interferons role in SVR with dominant genotype TT in Japanese patients (Asahina *et al.* 2014). In phase 2 clinical trials for DDAs patients with (rs12979860) CC genotype tended to achieve higher SVR as compare to other polymorphism (Zeuzem *et al.* 2013). Lazarevic and co-workers also founded the strong correlation

between (rs12979860) CC genotype and SVR in chronic HCV patients from Serbia (Lazarevic *et al.* 2013). Sarrazin and co-workers found similar results in 267 patients (Sarrazin *et al.* 2011). Several reports have indicated that there is a close relationship between polymorphism in rs12979860 and treatment outcomes to interferons plus ribavirin, higher response were observed in patients with CC, CT and TT polymorphism respectively (Aziz *et al.* 2015b) . The rapid virological response (RVR) has been achieved in chronically infected HCV patients regardless of any preferred genotype (Aziz *et al.* 2012). IL28B is responsible for the release of IFN- λ3 that has specific antiviral activity in the infected cells (Afdhal *et al.* 2011). In the present study correlation of rs12979860 polymorphism in the IL28B gene with response to IFN plus RBV was assessed in HCV-3a infected patients from slums of Islamabad. We found that patients with CC polymorphism at IL28B (rs12979860) have strong correlation with treatment response. Bota and colleagues also reported that SVR is significantly higher in the patients with CC polymorphism at IL28B (rs12979860) regardless of the regimens used for the treatment of chronic HCV infection (Bota *et al.* 2013).

Limitations of the study

The study presented here has several limitations. The patients were not of the same age and gender. Different hematological parameters such as hemoglobin and total leukocytes were not measured.

Conclusion

Among the viral and host genetic and other factors studied, only IL28B polymorphism was found to be correlated with SVR, genotyping of IL28B polymorphism is necessary in patient with chronic HCV before prescribing suitable antiviral therapy. This will help the physicians in prescribing suitable dose and treatment regimen.

Acknowledgements

Authors are thankful to the participants of the study for their cooperation. Financial support from Higher Education Commission (HEC) of Pakistan and Department of Biotechnology, Quaid-i-Azam University, Islamabad, for providing the basic facilities to conduct the study are highly acknowledged. AZ is supported by indigenous PhD fellowship from HEC.

References

Afdhal NH, McHutchison JG, Zeuzem S, Mangia A, Pawlotsky JM, Murray JS, Shianna KV, Tanaka Y, Thomas DL, Booth DR. 2011. Hepatitis C pharmacogenetics: state of the art in 2010. Hepatology **53(1),** 336-345.

Akbar H, Idrees M, Manzoor S, Ur Rehman I, Butt S, Yousaf M, Rafique S, Awan Z, Khubaib B, Akram M. 2009. Hepatitis C virus infection: A review of the current and future aspects and concerns in Pakistan. Journal of General and Molecular Virology **1(2),** 012-018.

Ali M, Afzal S, Zia A, Hassan A, Khalil AT, Ovais M, Shinwari ZK, Idrees M. 2016. A systematic review of treatment response rates in Pakistani hepatitis C virus patients; current prospects and future challenges. Medicine **95(50)**, e5327.

Asahina Y, Tsuchiya K, Nishimura T, Muraoka M, Suzuki Y, Tamaki N, Yasui Y, Hosokawa T, Ueda K, Nakanishi H. 2014. Genetic variation near interleukin 28B and the risk of hepatocellular carcinoma in patients with chronic hepatitis C. Journal of gastroenterology **49(7),**1152-1162.

Aslam K. 2016. Pakistan: "When they are diagnosed with hepatitis C, patients think they will die". Médecins Sans Frontières.

Asselah T, De Muynck S, Broet P, Masliah-Planchon J, Blanluet M, Bieche I, Lapalus M, Martinot-Peignoux M, Lada O, Estrabaud E. 2011. IL28B polymorphism is associated with treatment response in patients with genotype 4 chronic hepatitis c; wiley-blackwell commerce place, 350 main st, malden 02148, ma USA. p 868A-868A.

Asselah T, De Muynck S, Broët P, Masliah-Planchon J, Blanluet M, Bièche I, Lapalus M, Martinot-Peignoux M, Lada O, Estrabaud E. 2012. IL28B polymorphism is associated with treatment response in patients with genotype 4 chronic hepatitis C. Journal of hepatology **56(3),** 527-532.

Aziz H, Gil ML, Waheed Y, Adeeb U, Raza A, Bilal I, Athar MA. 2011. Evaluation of prognostic factors for Peg Interferon alfa-2b plus ribavirin treatment on HCV infected patients in Pakistan. Infection, Genetics and Evolution **11(3),** 640-645.

Aziz H, Raza A, Ali K, Khattak JZ, Irfan J, Gill ML. 2015a. Polymorphism of the IL28B gene (rs8099917, rs12979860) and virological response of Pakistani hepatitis C virus genotype 3 patients to pegylated interferon therapy. International journal of infectious diseases: IJID: official publication of the International Society for Infectious Diseases **30,** 91-97.

Aziz H, Raza A, Ali K, Khattak JZK, Irfan J, Gill ML. 2015b. Polymorphism of the IL28B gene (rs8099917, rs12979860) and virological response of Pakistani hepatitis C virus genotype 3 patients to pegylated interferon therapy. International Journal of Infectious Diseases **30,** 91-97.

Aziz H, Raza A, Waheed Y, Gill U, Gill ML. 2012. Analysis of variables and interactions among variables associated with a sustained virological response to pegylated interferon alfa-2a plus ribavirin in hepatitis C virus genotype 3-infected patients. International Journal of Infectious Diseases **16(8),** e597-e602.

Bota S, Sporea I, Şirli R, Neghină AM, Popescu A, Străin M. 2013. Role of interleukin-28B polymorphism as a predictor of sustained virological response in patients with chronic hepatitis C treated with triple therapy: a systematic review and meta-analysis. Clinical drug investigation **33(5),** 325-331.

Cieśla A, Bociąga-Jasik M, Sobczyk-Krupiarz I, Głowacki MK, Owczarek D, Cibor D, Sanak M, Mach T. 2012. IL28B polymorphism as a predictor of antiviral response in chronic hepatitis C. World Journal of Gastroenterology: WJG **18(35),** 4892.

Cooper S, Erickson AL, Adams EJ, Kansopon J, Weiner AJ, Chien DY, Houghton M, Parham P, Walker CM. 1999. Analysis of a successful immune response against hepatitis C virus. Immunity **10(4),** 439-449.

Dalgard O, Bjøro K, Hellum KB, Myrvang B, Ritland S, Skaug K, Raknerud N, Bell H. 2004. Treatment with pegylated interferon and ribavarin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. Hepatology **40(6),** 1260-1265.

De Nicola S, Aghemo A, Grazia Rumi M, Galmozzi E, Valenti L, Soffredini R, De Francesco R, Prati GM, D'Ambrosio R, Cheroni C. 2012. Interleukin 28B polymorphism predicts pegylated interferon plus ribavirin treatment outcome in chronic hepatitis C genotype 4. Hepatology **55(2),** 336-342.

El-Zanaty F, Way A. 2009. Egypt Demographic and Health Survey, 2008. Ministry of Health, El-Zanaty and Associates, and Macro International, Cairo Egypt.

Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, El Khoby T, Abdel-Wahab Y, Anwar W, Sallam I. 2000. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. The Lancet **355(9207),** 887-891.

Fried MW. 2002. Side effects of therapy of hepatitis C and their management. Hepatology **36(S1)**.

Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ. 2009. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature **461(7262),** 399-401.

Idrees M. 2008. Development of an improved genotyping assay for the detection of hepatitis C virus genotypes and subtypes in Pakistan. Journal of virological methods **150(1),** 50-56.

Idrees M, Riazuddin S. 2008. Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. BMC infectious diseases **8(1),** 1.

Kau A, Vermehren J, Sarrazin C. 2008. Treatment predictors of a sustained virologic response in hepatitis B and C. Journal of hepatology **49(4),** 634-651.

Khairy M, Fouad R, Mabrouk M, El-Akel W, Awad AB, Salama R, Elnegouly M, Shaker O. 2013. The impact of interleukin 28b gene polymorphism on the virological response to combined pegylated interferon and ribavirin therapy in chronic HCV genotype 4 infected egyptian patients using data mining analysis. Hepatitis monthly **13(7)**.

Khan T, Qazi J. 2013. Hurdles to the global antipolio campaign in Pakistan: an outline of the current status and future prospects to achieve a polio free world. Journal of epidemiology and community health **67(8),** 696-702.

Lazarevic I, Djordjevic J, Cupic M, Karalic D, Delic D, Svirtlih N, Simonovic J, Svorcan P, Milic N, Jovanovic T. 2013. The influence of single and combined IL28B polymorphisms on response to treatment of chronic hepatitis C. Journal of Clinical Virology **58(1),** 254-257.

Mangia A, Thompson AJ, Santoro R, Piazzolla V, Tillmann HL, Patel K, Shianna KV, Mottola L, Petruzzellis D, Bacca D. 2010. An IL28B polymorphism determines treatment response of hepatitis C virus genotype 2 or 3 patients who do not achieve a rapid virologic response. Gastroenterology **139(3),** 821-827. e821.

McCarthy JJ, Li JH, Thompson A, Suchindran S, Lao XQ, Patel K, Tillmann HL, Muir AJ, McHutchison JG. 2010. Replicated association between an IL28B gene variant and a sustained response to pegylated interferon and ribavirin. Gastroenterology **138(7),** 2307-2314.

McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, Nyberg LM, Lee WM, Ghalib RH, Schiff ER. 2009. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. New England Journal of Medicine **361(6),** 580-593.

Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. 2015. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology **61(1),** 77-87.

Moghaddam A, Melum E, Reinton N, Ring-Larsen H, Verbaan H, Bjøro K, Dalgard O. 2011. IL28B genetic variation and treatment response in patients with hepatitis C virus genotype 3 infection. Hepatology **53(3),** 746-754.

Moreira S, Garcia RF, Gutberlet A, Bertol BC, Ferreira LE, Pinho Mde S, de Franca PH. 2012. A straightforward genotyping of the relevant IL28B SNPs for the prediction of hepatitis C treatment outcome. Journal of virological methods **184(1-2),** 93-97.

Ohno O, Mizokami M, Wu R-R, Saleh MG, Ohba K-i, Orito E, Mukaide M, Williams R, Lau J. 1997. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. Journal of clinical microbiology **35(1),** 201-207.

Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi E, Borovicka J. 2010. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology **138(4),** 1338-1345. e1337.

Rehermann B, Nascimbeni M. 2005. Immunology of hepatitis B virus and hepatitis C virus infection. Nature Reviews Immunology **5(3),** 215-229.

Sarrazin C, Susser S, Doehring A, Lange CM, Müller T, Schlecker C, Herrmann E, Lötsch J, Berg T. 2011. Importance of IL28B gene polymorphisms in hepatitis C virus genotype 2 and 3 infected patients. Journal of hepatology **54(3),** 415-421.

Shaala AY, Morsi MG, Harfoush RAH, ElKhouly EH, Ahmed MAR, Roshdy MN. 2015. Role of IL-28B polymorphisms in virologic response to combined pegylated interferon and ribavirin therapy in genotype 4 chronic HCV infected patients with and without cirrhosis. Alexandria Journal of Medicine **51(3),** 231-239.

Shepard CW, Finelli L, Alter MJ. 2005. Global epidemiology of hepatitis C virus infection. The Lancet infectious diseases **5(9),** 558-567.

Shinwari ZK, Khalil AT, Nasim A. 2014. Natural or deliberate outbreak in Pakistan: how to prevent or detect and trace its origin: biosecurity, surveillance, forensics. Archivum immunologiae et therapiae experimentalis **62(4),** 263-275.

Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E. 2009. IL28B is associated with response to chronic hepatitis C interferon-α and ribavirin therapy. Nature genetics **41(10),** 1100-1104.

Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S. 2009. Genome-wide association of IL28B with response to pegylated interferon-α and ribavirin therapy for chronic hepatitis C. Nature genetics **41(10),** 1105-1109.

Wohnsland A, Hofmann WP, Sarrazin C. 2007. Viral determinants of resistance to treatment in patients with hepatitis C. Clinical microbiology reviews **20(1),** 23-38.

Zeuzem S, Soriano V, Asselah T, Bronowicki J-P, Lohse AW, Müllhaupt B, Schuchmann M, Bourlière M, Buti M, Roberts SK. 2013. Faldaprevir and deleobuvir for HCV genotype 1 infection. New England Journal of Medicine **369(7),** 630-639.