

**Role of Interleukin-10 polymorphism in  
determining the outcome of interferon therapy  
on Hepatitis C patients infected with genotype 3a**



**By**

**Talha Shafi**

**2008-NUST-BS-V&I-03**

**Atta-ur-Rehman School of Applied Biosciences  
National University of Science & Technology  
Islamabad, Pakistan  
2012**

**Interleukin-10 polymorphism in determining  
the outcome of interferon therapy on Hepatitis  
C patients infected with genotype 3a**

**By  
Talha Shafi  
2008-NUST-BS-V&I-03**

A thesis submitted in partial fulfillment of the requirement for  
the degree of Bachelors of Applied Biosciences  
With  
Majors in Immunology

**Atta-ur-Rehman School of Applied  
biosciences National University of Science &  
Technology Islamabad, Pakistan  
2012**



# National University of Sciences & Technology

FORM TH-1

## BS (Hons). THESIS WORK

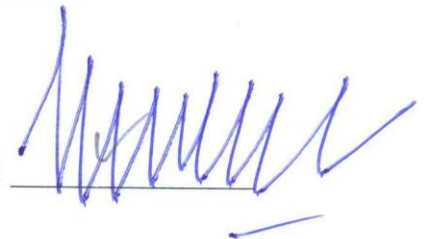
We hereby recommend that the thesis prepared by Mr. Talha Shafi Titled:  
**Role of Interleukin-10 polymorphism in determining the outcome of  
interferon therapy on Hepatitis C patients infected with genotype 3a**, be  
accepted in its present form to satisfy the thesis requirement of BS (Hons)  
degree.

### Guidance & Examination Committee Members

#### Thesis supervisor:

- 1) Dr M. Ashraf
- 2) Professor/Principal  
ASAB, NUST

Signature: \_\_\_\_\_



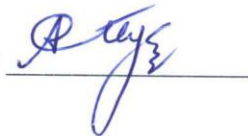
#### Thesis Committee Members

- 1) Dr Touqeer Ahmed  
Asst. Professor (Immunology)  
ASAB, NUST
- 2) Dr Attiya Bhatti  
Asst. Professor (Genomics)  
ASAB, NUST

Signature: \_\_\_\_\_



Signature: \_\_\_\_\_



**Dr. Muhammad Ashraf**  
Professor/Principal  
Atta ur Rahman School of Applied  
Biosciences NUST Department

27-06-2012

Date

#### COUNTERSIGNED

Date: 27-06-2012

Date: 27-06-2012

NUST Atta –ur- Rahman School of Applied Biosciences (ASAB), H-12  
Islamabad

## **ACKNOWLEDGEMENTS**

All the prayers to the All Mighty Allah who's blessing were with me through all these years. I want to convey my profound gratitude to my family whose support and love enabled me to live a thriving life.

I want to thank Mr M. Ashraf (Principle: Atta ur Rehman School of Applied sciences) who's guidance and financial Support encouraged me to work with full devotion and enthusiasm on this project. As a NUST student, I thank NUST to provide me the platform to excel in the field.

I am grateful to my supervisor and my mentor Mrs Sadia Tahir whose devotion has been a source of inspiration and dedication for me. I also want to thank Mr Sohail Afzal who was always there to support me in my work. I want to thank Sir Tahir A. Baig to encourage me to attain my goals. Especially my lab fellows: Ms Faryal Mehwish Awan and Mrs Bayyanah Adnan for their continuous support and affection.

I am very grateful to Dr Faraz Ahmed Bhatti and Ms Sadia Altaf for spearing time and reviewing my manuscript.

# *Dedication*

**I dedicate my work to**

**My parents,**

**My brothers**

**And**

**All those who supported me**

## **TABLE OF CONTENTS**

Chapters	Page no
Acknowledgement.....	iv
Table of Contents.....	vi
List of Abbreviations.....	vii
List of figures.....	x
List of tables.....	xi
Abstract.....	xii
1. Introduction.....	1
2. Review of Literature.....	5
2.1. Interferon .....	5
2.1.1 Function of interferon.....	6
2.1.2 Interferon signaling pathway.....	7
2.2. Hepatitis C.....	9
2.3. Interferon therapy.....	10
2.3.1. Factor effecting interferon therapy.....	10

2.4. Interleukin-10.....	12
2.5. IL-10/INF paradigm and Th1/Th2 regulation.....	15
2.6. Ethnicity and cytokine gene polymorphism.....	17
2.7. Genetic variation and Sustain virological responses...	17
3. Materials and Methods.....	19
3.1. Patients enrolment.....	19
3.2. Sample collection.....	19
3.3. Genomic DNA extraction.....	19
3.4. Genetic Analysis .....	20
3.4.1. Primers.....	21
3.4.2. PCR reaction mixture.....	22
3.5. Gel electrophoresis.....	24
3.6. Statistical Analysis.....	24
4. Results.....	25
5. Discussion.....	35
6. References .....	40



## **LIST OF ABBREVIATIONS**

~	Approximate
%	Percentage
GT	Genotype
ARMS	Amplification Refractory Mutation System
CD	Cluster of differentiation
DNA	Deoxyribonucleic acid
HCV	Hepatitis C virus
IFN- $\alpha$	Interferon - $\alpha$
IFN- $\gamma$	Interferon - $\gamma$
IL-2	Interleukine-2
IL-4	Interleukine-4
IL-5	Interleukine-5
IL-6	Interleukine-6
IL-10	Interleukine-10
IL-13	Interleukine-13
NF $\kappa$ B	Nuclear factor kappa beta

kDa	Kilo Dalton
LPS	Lipopolysaccharide
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
SNP	Single nucleotide polymorphism
SOCS	Suppressor of cytokine signaling
STAT	Signal Transducers and Activators of Transcription
Th 1	T helper 1
Th 2	T helper 2
TNF	Tumor necrosis factor
SVR	Sustain virological response
NSR	Non sustained virological response

## LIST OF FIGURES

Figure	Title	Page no
Figure 2.1	Diagrammatic representation of two step model of interferon mechanism.	8
Figure 2.2	Schematic diagram of Th1/Th2 activation from Thp cell by interferons	16
Figure 3.1	Schematic diagram of PCR reaction	23
Figure 4.1	Representative gel picture of samples Heterozygous for both alleles	25
Figure 4.2	Representative gel for sample Homozygous for one Allele	26
Figure 4.3	Graphical representation of percentage frequency of genotype in HCV and control group	29
Figure 4.4	Graphical representation of %age frequency of IL-10 genotype in SVR and NSR groups	31
Figure 4.5	Haplotype frequency in SVR and NSR groups	34

## **LIST OF TABLES**

Table	Title	Page no
Table 3.1	List of primers	21
Table 3.2	List of ingredients and concentrations used in reactions	22
Table 4.1	Comparison between baseline properties of the samples	27
Table 4.2	Comparisons of IL-10 genotype/allele frequency in healthy controls and chronic hepatitis C patients	28
Table 4.3	Comparison of IL-10 genotype/allele frequency in responders and non-responders	30
Table 4.4	Comparison of IL-10 haplotype in healthy and C-HC patients	32
Table 4.5	Comparison of IL-10 haplotypes in responder and non-responders	33

## **ABSTRACT**

Pakistan is a low socio economic country having more than 10 million people infected with hepatitis C Virus (HCV) with a major genotype of 3a (GT 3a). Due to high rate of resistance to standard interferon plus ribavirin therapy, it is highly needed to identify new marker for response prediction to therapy. Interleukin 10 (IL-10) is a key member of cytokine, which regulates Th1/Th2 cytokine balance, a major part of immune system against infection. As IL-10 production varies interindividually based on some functional polymorphism in its promoter region. We studied the impact of functionally important polymorphism (-1082 G/A, -819 C/T and -592 C/A) on HCV infection susceptibility and on outcomes of standard interferon plus ribavirin therapy. 90 healthy subjects and 140 HCV patients (95 were responder and 45 were non-responder to therapy) were included in this study. Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method was used for IL-10 polymorphism genotyping. High IL-10 producing -1082 GG genotype ( $p= 0.02$ ; OR= 0.4; 95% CI= 0.2-0.8) and GTA haplotype ( $p=0.03$ ; OR= 0.55; 95% CI= 0.3-1) were significantly higher in HCV patients as compared to healthy subjects. IL-10 -1082 GA genotype ( $p=0.03$ ; OR= 1.95; 95% CI= 1.1-3.4) showed protective effect against HCV infection while other allelic, genotypic and haplotypic variants were nonsignificant among HCV patients compared with healthy controls. The current data failed to show any significant co relation between IL-10 polymorphism inheritance and therapy response in HCV patients. Our data showed a significant effect of IL-10 promoter gene polymorphisms and HCV infection susceptibility or protection but fail to demonstrate the influence of IL-10 promoter gene polymorphisms on the

response to combination therapy in Pakistani chronic hepatitis C patients infected with 3a genotype. The impact of genetic variations in IL-10 polymorphic variants on the response to anti-HCV treatment among different ethnic populations deserves further examination.

[PDF to Word](#)