

# Molecular Detection of Interleukin 28B Gene rs8099917 Polymorphism in chronic HCV Patients from Khartoum State, Sudan

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## ABSTRACT

**Background:** Hepatitis C virus (HCV) infection represents a major health burden. According to the World Health Organization (WHO) there are more than 170 million people infected worldwide and 3-4 million new infections are estimated per year. Recent studies have demonstrated the role of interleukin B 28 (IL 28B) polymorphism in predicting the treatment induced and spontaneous clearance from hepatitis C virus (HCV) infection and genome-wide association studies have shown that single nucleotide polymorphism (SNPs) near interleukin 28B gene are good predictors of response to treatment. The present study aimed to isolate and identify IL 28B gene rs8099917 polymorphism from HCV infected individuals DNA.

**Methods:** This cross-sectional study was performed on 50 blood samples from patients with chronic HCV infection as detected by ELISA kit (RIBA-11 and c-200/c-22 ELISA). DNA was extracted from the samples and the frequency of the polymorphism was analyzed by using PCR-RFLP method.

**Results:** The analysis of the data for G/T polymorphism showed that GT heterozygous was found in 14(28%) patients (10 males, 4 females), and TT homozygote was detected in 36(72%) patients (26 males, 10 females) and no GG homozygous genotype was detected.

**Conclusion:** In this study investigation of rs8099917 (T/G) Polymorphism in interleukin 28B gene (IL28B) in 50 HCV positive patients from Khartoum State indicated that the TT genotype was the dominant genotype detected.

**Keywords:** HCV Infection; Polymorphism; ELISA

## INTRODUCTION

Hepatitis C virus is considered as one of the most common causes of liver diseases worldwide and is the primary cause of chronic active hepatitis in 80% of the infected patients with the virus only eliminated in about 10%–20% of infected patients and more than 20%–30% of those chronically infected patient go on to develop progressive cirrhosis and hepatocellular carcinoma (HCC) especially if left un-treated or poorly treated [1,2].

Genome-wide association studies showed that genetic variations in the region near the interleukin-28B (IL28B) gene; on chromosome 19q13.13; which encodes interferon- $\lambda$ 3 (IFN- $\lambda$ 3) are associated with chronic HCV treatment response [3]. Interleukin 28B (IFN $\lambda$ 3) is

an immunodulatory cytokine which up regulates MHC class-I antigen expression; it possesses a potent antiviral as well as anti-tumor activity [4]. IL28B helps in clearance of the HCV by inhibiting its replication through Janus kinase/signal transducer and through activation of transcription pathway. In patients with chronic infection, an association between IL28B genotype and divergent expression of the intrahepatic IFN-stimulated genes was found [5]. Many investigations showed that genetic variations in IL28B SNP rs8099917 near the IL28B gene region are associated with clearance of HCV RNA in -HCV antibody positive individuals [3]. Many studies worldwide are now aimed to study and detect this polymorphism in Interleukin 28B gene rs8099917 [6-11].

The IL 28B gene rs8099917 polymorphism in HCV infected

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Sudanese patients in Khartoum state is unknown, therefore this study aimed to detect and identify this polymorphism.

## MATERIALS AND METHODS

### Study

This was a cross-sectional study applied on chronic HCV positive patients of over 16 years old during March to May 2017, from Elnao hospital Khartoum, Sudan. The information regarding date, time of recruitment, serials number, patient's code, sex, age, and residence were assembled from the hospital records.

### Collection of specimens

A 5 ml of peripheral blood were collected into EDTA container form HCV infected patients (n=50). Collected samples were transported to the Central laboratory-Ministry of higher Education; on ice bags and stored at -20°C until testing time.

### DNA extraction and rs8099917snp amplification

Genomic DNA was extracted from HCV infected patients by salting out method and the sequence of rs8099917 SNPs was amplified by PCR using the following primers:

Forward primer: 5'-CATCCCACCTTCTGGAACAAAT-3'

Reverse primer: 5'-GTATCAACCCACCTCAAATTATC-3' (12).

Cycling conditions composed of one cycle of initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 40 seconds, then final extension at 72°C for 2 minutes. The amplified product (400 bp) was identified on a 2% agarose gel [12].

### The SNP genotype identification

The rs8099917 SNP (T/G) genotyping was obtained by RFLP (restriction fragment length polymorphisms) using BseMI (BsrDI) endonuclease (Thermo Scientific, Lithuania). The total digestion reaction tubes contained 5 µL of qualified PCR product, [1X] buffer R and 1U of BseMI incubated at 55°C for two hours. The RFLP bands of interest were separated on 3.5% agarose gel stained with ethidium bromide for visualization on a UV transilluminator [12]. BseMI digestion results in fragment of 400 bp in case of genotype TT, fragments of 400 bp, 234 bp and 166 in case of genotype GT and fragments of 234 and 166 bp in case of GG polymorphism.

## RESULT

We successfully amplified the IL28B gene from the 50 sampled Sudanese patients (26 males and 24 females) who were chronically infected with HCV. Out of these 50 patients, 36 (72%) were carrying genotype TT [16 male (44.4%) and 20 females (55.6%)]

while 14 (28%) were GT heterozygous [10 male (71.4%) and 4 female (28.6)]. There was no HCV chronically infected patient carrying GG genotype (0%) (Table 1).

## DISCUSSION

Little is known about the IL28B rs 8099917SNP in HCV infected Sudanese patients. This current study is the first to detect and identify G/T polymorphism of IL28B gene rs 8099917 SNP in HCV chronically infected patients in Sudan.

The study found that IL28B rs8099917 SNP genotypes among the Sudanese HCV infected patients were the TT homozygote and GT heterozygote, while the homozygote GG was not detected and the dominant genotype among these patients is the TT genotype. Similar to our high TT genotype prevalence (72%), Baghbani et al., Ridruejo Aziz et al., Echeverria et al., and Kandoussi et al., found the TT genotype in 65%, 60%, 60%, 57.7% and 67% of the patients respectively [13-16]. In contrast, another study done by Tameshkel et al., genotype TT was reported in 25% of the tested population [17].

This study report GT genotype in 14 (28%) of HCV infected Sudanese patients, this result is in agreement with Aziz et al., Echeverria et al., and Kandoussi et al., who reported this genotype in 36.2%, 27.2% and 31.7% respectively [14-16]. Jordovic et al., report GT genotype in 78.6% of the tested population and this result disagree with these study findings [18].

GG genotypes was not detected in Sudanese patients tested in this study, Aziz et al., Kandoussi et al., and Sticchi et al., also found this genotype in low percentages 3.8% and 1% respectively while Echeverria et al., reported GG genotype in 14% of the tested population [11, 14-16]. Thus, performing the same study in large number of HCV patients from different areas of the country should be done to confirm the exact prevalence of the GG genotype in Sudanese patients.

Patients with genotypes TT rs8099917 are statistically more able to achieve sustained virologic response (SVR) to PEG-IFN/RBV in hepatitis C infected patients compared to patients carrying the G allele of rs8099917 (TG and GG) and the T allele of rs12979860 (TT and GT) [19]. SNPs in rs8099917 gene IL28B might also be associated with risk of Chronic Infection by HCV but not with response to treatment [20]. Further studies are thus needed to determine the relation of the genotypes detected and response to treatment and infection outcome in Sudanese HCV patients.

Finally this obtained result should call for a wider surveillance at the national level in order to elucidate the true status of HCV infection and that of the Interleukin 28b gene rs 8099917, as well as other alleles polymorphisms and genes which were found to affect spontaneous viral clearance and response to HCV treatment in both HCV patients and the general population.

**Table 1:** Particulars of HCV patients and their IL28B gene rs8099917 genotypes.

	Male	Female	Total
NO	26 (52%)	24(48%)	50
Age mean ± SD	35.5 (Range 17-60 year)	33.3 (Range 19-52 year)	
TT genotype	16(44.4%)	20(55.5%)	36(72%)
GT genotype	10 (71.4%)	4(28.6%)	14(28%)
GG genotype	-	<2%	-

## CONCLUSION

In this study investigation of rs8099917 (T/G) polymorphism in interleukin 28B gene (IL28B) in 50 HCV positive patients from Khartoum State indicated the presence of only two genotypes (TT and TG) with the TT being the dominant genotype detected. Homozygous GG genotype was not detected in any of the samples.

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