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HUMAN GENETIC VARIATION SUSCEPTIBILITY ASSOCIATED WITH HBV: ANALYSIS OF GENETIC MARKERS SUSCEPTIBILITY TO HEPATITIS B AMONG PATIENTS IN BABYLON PROVINCE

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ABSTRACT

Background: Hepatitis is a serious public health problem distressing many of people worldwide. Inadequate data is available on this issue in Iraq. This study was carry out with the aim of determining the genetic SNPs in human and risk factors of hepatitis C virus (HCV) among the general population and among blood donors. **Methods:** Blood samples from volunteers; have been screened with ELISA tests for detecting the hepatitis- surface antigen and using PCR for the same purpose. In addition we used some genes to study the prevalence of hepatitis severity with human genes polymorphism like IFNGR, IFN-γ and IL-28B gene. **Results:** The characteristics of the 248 patients included in the study are described in with criteria including, Age as Characteristics feature, Residence type, Risk factor and Gender. Polymorphisms in the HBV patients revealed the presence of the polymorphism in IFNGR gene at chromosome 6 in position 137219896+137219995 among 47% of patients compare with healthy group 21%. Study of HBV with healthy group against other test genes revealed a marked significance in the IFN-γ genes. There was a strong association of the IFN-γ at chr12:68158673-68158792 with HBV compare with healthy controls 61.4% and 27.08% respectively. Profile of gene Expression with IL28B Genotype revealed that (19.6%) patients with CC genotype, (71.6%) with CT (patients) and patients (8.8%) with TT genotype among chronic viral hepatitis B patients. **Conclusions:** Our study provided much important information concerning hepatitis B prevalence and risk factors.

KEYWORDS: Iraq, Hepatitis C, Prevalence, General population, Risk factors, Blood donors, CD4+ and CD8+, IFNGR, IFN-γ, IL-28B gene.

INTRODUCTION

Hepatitis B (HBV) together with hepatitis C (HCV) accounts for 75% of liver diseases and are regarded a major threat to public health worldwide.[1,2] Hepatitis B and C co-infections have raised major concern in HIV, transplant and other immunosuppressed patients. Intravenous drug abuse is currently the main risk but nosocomial infection is also of concern for HBV and HCV infection.[3,4,5] Three independent factors seem to be associated with fibrosis: age, daily alcohol consumption and male gender. Over two billion people are expected to be infected with HBV during their lifetime and about 350 million are estimated to be chronic carriers.[6] Acute HCV infection is asymptomatic in the majority of patients, but persists in about 70% of them.

There are many barriers help to decrease the severity of hepatitis infection like physical barriers, innate immunity comprises soluble components (e.g., complement factors, type I interferons) and cellular components (e.g., granulocytes, macrophages, dendritic cells, natural killer (NK) cells). Adaptive immunity includes humoral components (antibodies produced by B cells) and, especially important in viral infections, cellular immune responses (CD4+ and CD8+ T cells). Studies in humans and animal models of HCV and HBV infection have demonstrated that HCV elicits innate immune responses early after infection. However, the virus can persist in the face of the innate immune response. Indeed, viral clearance occurs only in the presence of antiviral CD4+ and CD8+ T cell responses.[7, 8]

A successful T cell response requires the presentation of viral peptides bound to HLA molecules on the surface of antigen-presenting cells to T cells bearing a reactive T cell receptor (TCR). Importantly, HLA alleles are extremely variable in the human population and several HLA types have been identified that are associated with different outcomes of HCV infection, the most prominent one being the protective HLA-B27 allele. Major histocompatibility complex (MHC) gene products are vital in regulating several antiviral immune reactions. In addition, genetic factors controlling host immune response could play an important role in determining infection outcome.

Alloantigens are taken up by antigen-presenting cells, which process them and re-express the antigens on the cell surface along with HLAs to be recognized by the T-cell receptor. The

polymorphisms of HLA alleles may cause significant changes in the presentation of antigen to T cell receptor, which in turn affects immune response.[9, 10] Several proinflammatory cytokines such as T helper (Th) 1 cytokines (including interleukin (IL)-2 and interferon (IFN)-gamma) have been identified to participate in the process of viral clearance and host immune response to HBV. In contrast, the Th2 cytokine (IL-10) serves as a potent inhibitor of Th1 effector cells in HCV diseases. Polymorphisms in the regulatory regions of the cytokine genes may influence their expression. Therefore, as genetic predictors of disease susceptibility or clinical outcome, the polymorphisms of cytokine genes are potentially important.[11, 12] The intracellular antiviral responses induced by IFN-γ play a critical role in the pathogenesis of HBV and HCV infection.[13] Upon induction of the signaling pathway, IFN-γ functions in an autocrine manner to up-regulate the transcription factor interferon regulatory factor 1 (IRF-1) and its target genes.[14,15] Subsequently, a feedback loop in which IFN-g and IRF-1 are upregulated results in the amplification of the IFN-g-dependent response to lipopolysaccharides (LPS). The importance of IFN-g for the clearance of HBV is well known.[16] It has been shown that IFN-γ can inhibit the replication of HBV-infected cells, directly reducing viral load by mediating the antiviral effect of the cytotoxic lymphocytes (CTLs).[17]

MATERIALS & METHODS

Subjects

Blood samples were collected from 248 patients whom diagnosed as having chronic hepatitis and were attending the outpatient clinic of central Health Laboratory at Al-Hillah Educational Hospital; also 70 normal subjects were examined as a control group. The patients were 47.2% males and females were 52.8%.

Patients and controls: Personal history, family history, previous liver disease, and gastrointestinal disturbance were taken. Complete clinical and abdominal examination for liver, spleen and gastrointestinal tract. Diagnosis for chronic hepatitis B for seropositivity and High levels of serum transaminases activity. Also Detection of hepatitis C markers by using the enzyme immunoassay kits (Acone) which included hepatitis C and immunoglobulin M to hepatitis C antigen followed manufactures instructions.

Detection of HBV Biomarkers

The populations selected in Al Hillah city. There were 382 people selected and there were only 248 cases with HBV, of whom 35.9% were from the rural population while 64.1% was

urban resident. There were 47.2% males and 52.8% females, in the age range of <15 to >45 years as showed in figure 1. The positive results were screened by ELISA.

DNA extraction Single nucleotide polymorphism genotyping

Genomic DNA was extracted from 300 ml of a peripheral whole blood sample using a commercially available DNA isolation kit (Invetrogen, USA) in accordance with the manufacturer's instructions. Single nucleotide polymorphisms (SNPs) of polymorphic genes were assessed in all study subjects: IFN-γ gene at position +874, and IFNGR-1 at 611 as shown in table 1. The sequencing primers were designed from the National Center for Biotechnology Information (NCBI) sequence data.[18] The thermocycling parameters for genes were as follows: an initial activation step of 95 ^{o}C for 10 min preceded the cycling program, followed by 35 cycles of denaturation at 95 ^{o}C, annealing at 72 ^{o}C for 1 min, and final extension at 72 ^{o}C for 7 min.

Table 1: PCR amplifying primers sequences

Gene	Forward	Reverse	bp
IFNGR	**5-acgttggatgcttctcagcaattcagtgtc-3**	**5-acgttggatgcaaacccagagaggtaagag-3**	100
IFN-γ	**5-atattcagacattcacaattgatt-3**	**5-tattattatacgagctltaaaagatagttcc-3**	120

For IFNGR gene, chr6:137219896+137219995 100bp, **IFN-γ** and chr12:68158673-68158792 120bp.

On the way to identify genotype incidence of polymorphism in IL-28B gene the molecular biology methods was used by classical DNA separation from blood samples then amplification by PCR, genotype was defined as CC, CT and TT type. The results were shown in picture 2.

It was used for that purpose IL28B rs17/rs60 Real-TM, from Sacace (Italy). Interleukin-28 (IL28) is a cytokine that plays a role in immune defense against viruses. IL28B belongs to the type III interferon family of cytokines. Its classification as interferon is due to its ability to induce an antiviral state.

Polymorphisms in the IL28B gene region are important in predicting outcome following therapy for chronic hepatitis C virus (HCV) infection. Combined therapy INF pegylated (PEG-IFN) and ribavirin (RBV) is the current standard therapy against HCV infection and to know in detail the polymorphism in IL28B gene region of patients infected with HCV can be

an important component of the decision to initiate treatment with PEGIFN and RBV. In particular, the rs12979860 polymorphism in the promoter of the IL28B human gene is strongly associated to the SVR (Sustained Viral Response).

It has been shown that patients who have the C/C genotype (genotype associated with favorable response to standard therapy) have a higher SVR rate compared to patients with genotype C/T and T/T (genotypes associated with less favorable response). The rs8099917 polymorphism in the promoter of the IL28B human gene is associated with the success or failure of treatment in patients with viral genotype HCV1. Amplification done as following:

Table 2: PCR amplifying condition with Rotor-Gene 6000/Q (Qiagen)

Step	Rotor type instruments1		
	Temperature °C	Time	Cycles
Hold	95	15 min	1
Cycling	95	5 s	5
	60	20 s	
Cycling 2	95	5 s	40
	60	40 s Fluorescence detection	

RESULTS AND DISCUSSION

Patient Characteristics; the characteristics of the 248 patients included in the study are described in Figure 1, 2, 3 and 4. Patients represented multiple criteria including, Age as Characteristics feature, Residence type, Risk factor and Gender.

Gene Expression Profile Versus IFN-γ and IFNGR Genotype; Evaluation of the incidence distribution of the various dimorphic polymorphisms in the HBV patients revealed the presence of the polymorphism in IFNGR gene at chromosome 6 in position 137219896+137219995 among 47% of patients compare with healthy group 21%. Study of HBV with healthy group against other test genes revealed a marked significance in the IFN-γ genes. There was a strong association of the **IFN-γ** at chr12:68158673-68158792 with HBV compare with healthy controls 61.4% and 27.08% respectively. It's showed higher frequency distributions in disease subjects than in healthy groups. Immune response have ability to control the preliminary infection determines the clinical consequence. HBV infection persistent was considered a multifactorial disorder with environmental, viral and genetic components. It is well known that the major mode of infection in HBV-endemic areas, including Korea, is perinatal transmission, but this is not the sole factor that can explain the

chronicity of HBV after acute infection.[19, 20] There are many studies; mainly in animal revealed that expression of many cytokine can intermediate decrease life cycle and replication of HBV in the absence of significant hepatocyte killing.[21] This appeared to involve IFN-γ and TNF-α. Most important cytokine is a glycoprotein with pleiotrophic (IFN-γ) which can reveal essential role in encouraging immune responses.[22] Recently it was discovered the importance of IFN- γ for the clearance of HBV, many reports revealed that the antigen-specific fraction of T cells in acute self-limited HBV infection selectively secrete Th1-type cytokines, with a predominance of IFN-γ.[23] IFN-γ can inhibit the replication of HBV infected cells, directly reducing viral load, moreover, IFN-γ mediated most of the antiviral effect of the CTLs. Other studies have revealed that the IFN-γ and TNF-a genes are related to the susceptibility to persistent HBV infection or HBV clearance.[24, 25] The results were shown in picture 1.

Profile of gene Expression with IL28B Genotype; According to previous reports suggesting modest differences between CT and TT genotypes at rs12979860 in relation to HBV genotypes were collapsed into a recessive model and comparisons were performed as CC versus CT or TT genotypes. A total of 248 genes were found to be differentially expressed between genotype groups. There were (19.6%) patients with CC genotype, T (71.6% patients) with C and patients (8.8%) with TT genotype among chronic viral hepatitis B patients as showed in figure 5 and 6.

It was inspected the association between IL28B genotype and HBV infection[26, 27], IL28B polymorphism related to variance gene expression profiles, where the worthy responses IL28B variants are associated with lower levels of hepatic expression. IL28B genotype was not associated with differences in intrahepatic IL28B gene expression, and protein sequence variants of IFN-k3 do not appear to explain the differences in ISG expression or anti-HCV response by IL28B genotype.[28, 29, 30]

In this study, there are significant association between IFN-γ, IFNGR, and IL-28B SNPs and susceptibility to persistent HBV infection.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

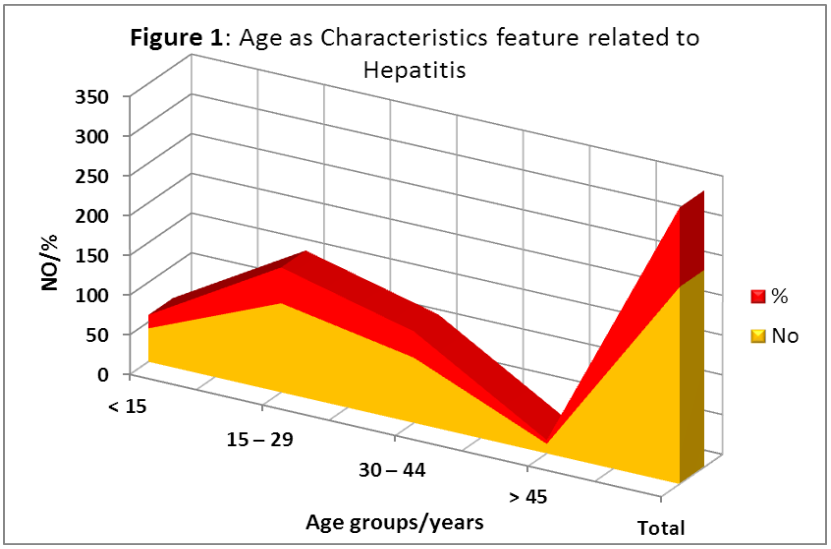


Figure 1: Age as Characteristics feature related to Hepatitis
350
300
250
200
150
100
50
0
NO/%
< 15
15 – 29
30 – 44
> 45
Total
Age groups/years
%
No

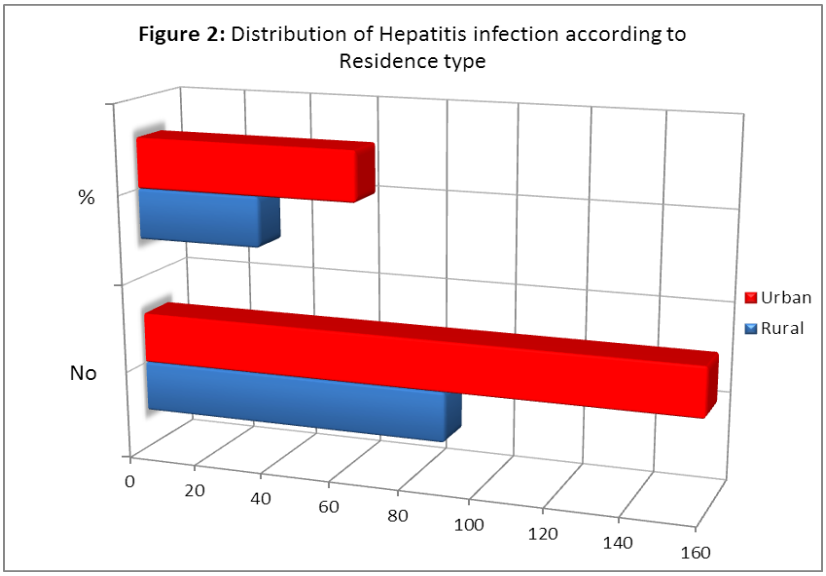


Figure 2: Distribution of Hepatitis infection according to Residence type
%
No
0
20
40
60
80
100
120
140
160
Urban
Rural

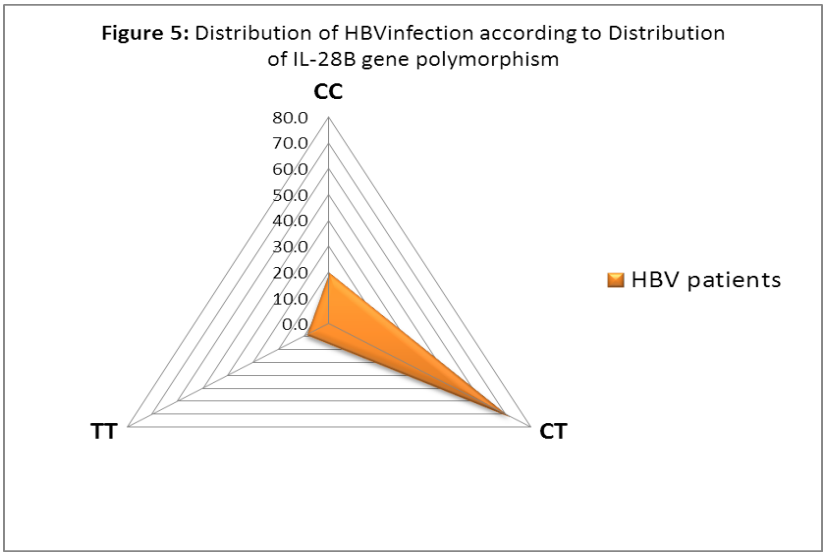


Figure 5: Distribution of HBVinfection according to Distribution of IL-28B gene polymorphism
CC
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
TT
CT
HBV patients

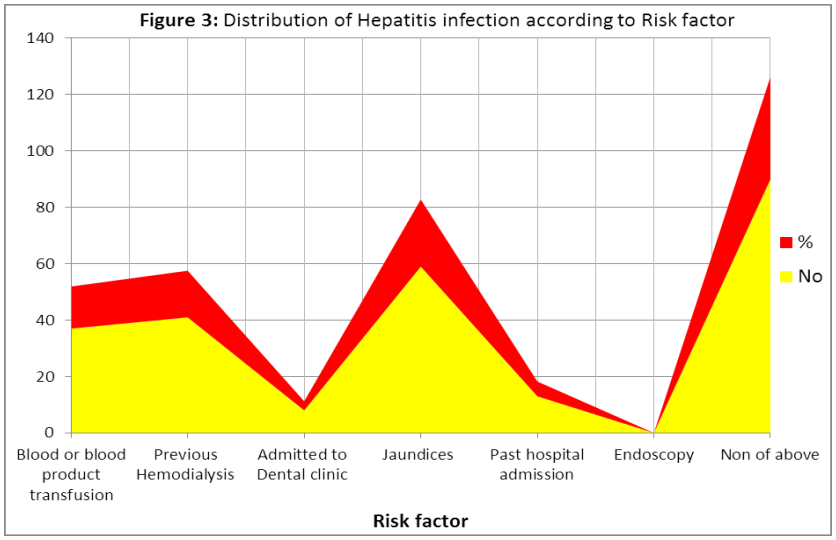


Figure 3: Distribution of Hepatitis infection according to Risk factor
140
120
100
80
60
40
20
0
%
No
Blood or blood product transfusion
Previous Hemodialysis
Admitted to Dental clinic
Jaundices
Past hospital admission
Endoscopy
Non of above
Risk factor

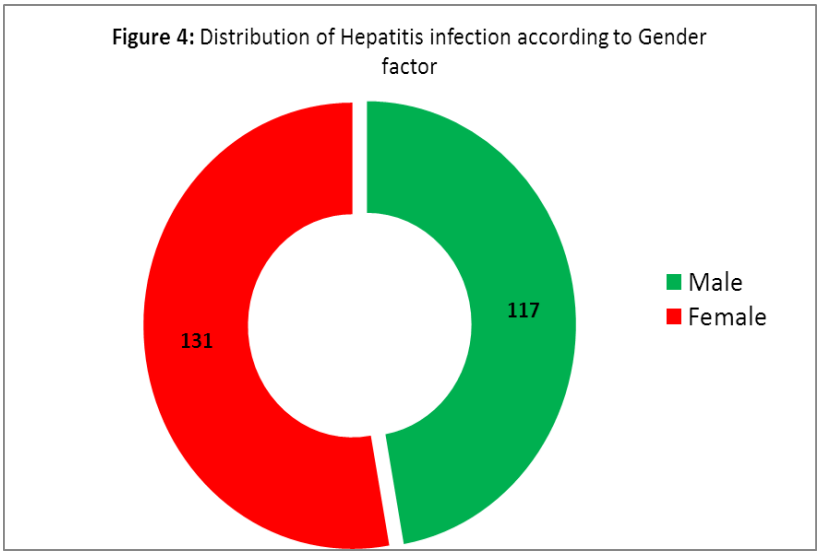


Figure 4: Distribution of Hepatitis infection according to Gender factor
117
131
Male
Female

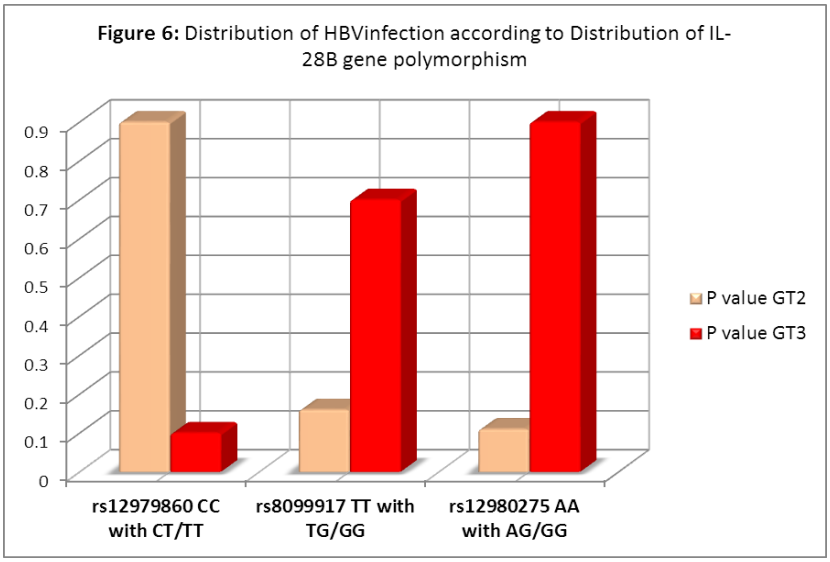
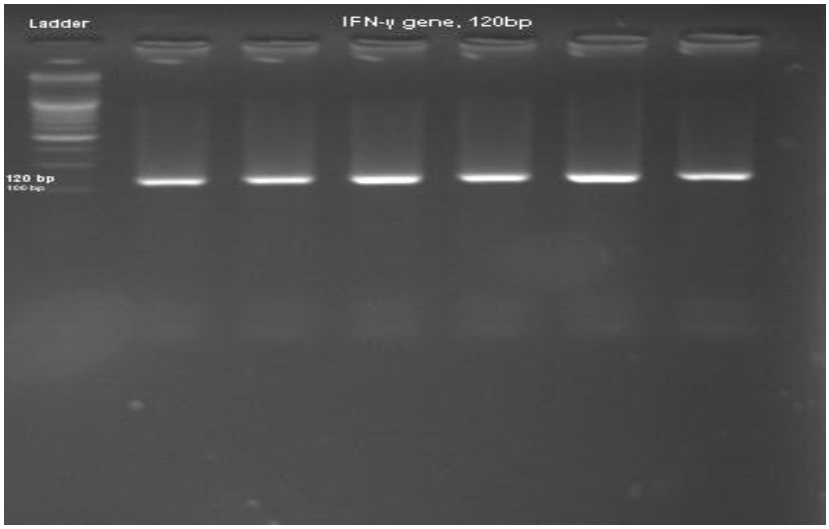


Figure 6: Distribution of HBVinfection according to Distribution of IL-28B gene polymorphism
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
P value GT2
P value GT3
rs12979860 CC with CT/TT
rs8099917 TT with TG/GG
rs12980275 AA with AG/GG



Picture 1: Genomic analysis of IFN-γ. The first line represent ladder (L) lanes contain the 100 bp DNA Step Ladder, 4% NuSieve® 3:1 agarose gel in 1X TBE buffer containing 0.8µg/ml ethidium bromide, lane 2-6 shows the Sample-DNA of IFN-γ (120 bp).

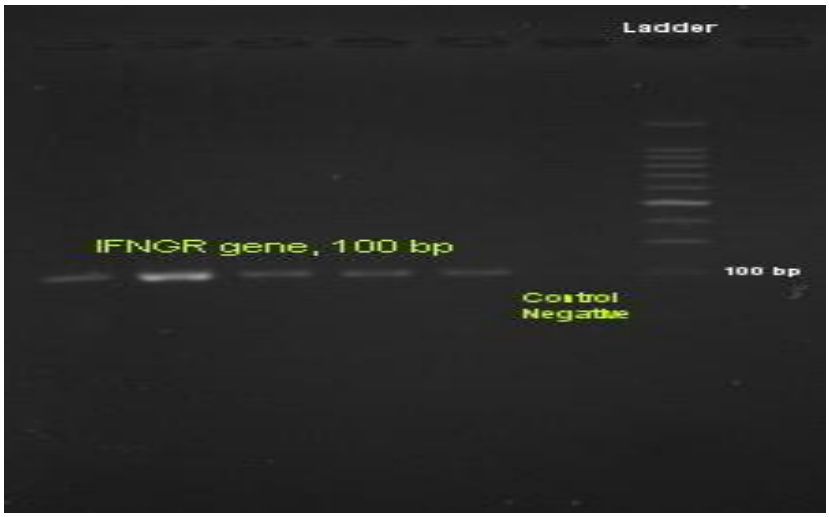


Figure 2: Genomic analysis of IFNGR. The last line represent ladder (L) lanes contain the 100 bp DNA Step Ladder, 4% NuSieve® 3:1 agarose gel in 1X TBE buffer containing 0.8µg/ml ethidium bromide, lane 2-5 shows the Sample-DNA of IFNGR (100 bp) while the 6th belong to negative control.

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