RESEARCH PAPER | Biochemistry | Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

Interleukin-1Beta and Tumor Necrosis Factor-Alpha Genes Polymorphism and Hepatocellular Carcinoma in Patients with Hepatitis C Virus in Egyptian Population

KEYWORDS: IL-1β, TNF-α, Gene polymorphism, HCV, HCC

Rizk El-Baz
Genetic Unit, Children Hospital, Mansoura University, Egypt

Amr El-Rabat
Internal Medicine Department, Faculty of Medicine, Mansoura University, Egypt

Mohamed Abd El-Moneim
Organic Chemistry Department, Faculty of Science, Port-Said University, Egypt.

*** Ghada awadalla**
Biochemistry Department, Faculty of Science, Port-Said University, Egypt.
* is Correspondent author

ABSTRACT *Background: Hepatitis C virus (HCV) infection is a major risk factor for developing HCC. Cytokines are involved in various cancers. This work aimed to investigate the relationship between the gene polymorphisms of interleukin-1 beta (IL-1β), IL-1 receptor antagonist (IL-1RN) and tumor necrosis factor alpha (TNF-α) and the development HCV-related HCC.*

Methods: The study included 100 HCV patients (50 with HCC and 50 without HCC) and 70 apparently healthy individuals. Gene polymorphisms of IL-1β–31 C/T, IL-1β–511 C/T, IL-1RN variable number of tandem repeats (VNTR), and TNF-α-308 G/A were investigated by PCR based assay.

Results: There were significant increase in the frequency of IL-1β–31 TT, IL-1RN A2A2 and TNF-α–308 AA genotypes in HCC group when compared to controls. There were, in addition, significant increase in the frequency of IL-1β-31 T, IL-1RN A2 and TNF-α-308 A alleles in HCC group when compared to controls. However, there was no significant difference in IL-1β–511 gene polymorphism between different groups.

Conclusion: HCV patients' carriers for IL-1β–31 T, IL-1RN A2 or TNF-α–308 A alleles could be at risk of developing HCC.

Introduction

Hepatitis C virus (HCV) infection is a global health problem, being the second most common chronic viral infection in the world with a global prevalence of about 3% (about 180 million people) (Craxi et al., 2008). Egypt is an endemic area of hepatitis C. It was found that about one person in seven of Egyptian population tested positive for antibodies against HCV. However, nearly one person in ten carries its viral RNA and is therefore chronically infected (Yehia et al., 2011). Patients infected with HCV have different clinical outcomes, ranging from acute resolving hepatitis to chronic liver disease including liver cirrhosis or hepatocellular carcinoma(HCC) (Zaltron et al., 2012). HCC is the fifth most common tumor and the third most common cause of cancer-related deaths worldwide (Okamoto et al., 2010). Its incidence has increased sharply over recent decades and this has been partially attributed to chronic HCV infection (Bosch et al., 2005). In Egypt, the growing incidence of HCC is nearly doubled over the last decade (Freedman et al., 2006). This is parallel with that Egypt is plugged with highest prevalence of HCV in the world, ranging from 6 to 28% (Khattab et al., 2010).

Cytokines, as the products of host response to inflammation, play an important role in the defense against viral infections. However, in HCV infection they may play a prominent role in liver damage (Koziel, 1999). Interleukin-1 beta (IL-1β) and tumor necrosis factor-α (TNF-β) are key cytokines in the inflammatory response (Dinarello, 1996). Interleukin 1 beta (IL-1β) is one of the potent pro-inflammatory cytokines and has a wide array of biological functions, including cell survival and proliferation (Roshak et al., 1996). Some single nucleotide polymorphisms (SNPs) have been reported in IL-1β, from which cytosine (C)/ thymidine (T) base transitions at –31 bp from the transcriptional start site. It has been shown that the –31T allele enhances IL-1β transcriptional activity (El-Omar et al., 2000) and a number of studies reported that –511C/–31T is a risk haplotype for the development of cancer (Chang et al., 2005). Another cytokine that has an important influence on IL-1β levels is the IL-1 receptor antagonist (IL-1RN); the gene encoding this cytokine; IL-1RN, is also known to be polymorphic (Santtila et al., 1998).

Circulating TNF-α level increases during HCV infection and hepatitis viral infection induces TNF-α production in human hepatocytes (Cua et al., 2007). An elevated TNF-α level correlates with the severity of hepatic inflammation, fibrosis, and tissue injury (Akpolat et al., 2005) Persistent immune mediated hepatic injury can initiate the process of fibrosis, cirrhosis, and, eventually HCC (Levrero, 2006). Polymorphism in the human TNF-α promoter at –308 (Wilson et al., 1992) involves the substitution of adenosine for guanine in the uncommon alleles. A variety of infectious diseases and inflammatory disorders are associated with TNF-α–308 alleles (Bdwell et al., 2001). The role of polymorphisms in TNF-α in the pathogenesis of HCV infection has been investigated, but some results are contradictory (Abbas et al., 2005). The aim of the present study was to determine the genotype and allele frequencies of IL-1β, IL-1RN and TNF-α polymorphisms among the Egyptian patients with chronic HCV infection (HCC and non-HCC) and healthy individuals to assess whether these genes are involved in chronic HCV susceptibility and/or HCC development.

Subjects and Methods

Patient group:

The study included 170 subjects; 100 patients attending Oncology Center - Mansoura University HCV (50 patients with HCC and 50 patients without HCC) and 70 healthy individuals with no liver affection (mean age/years ± SD; 55.24±5.72). The patients were subjected to full history taking and thorough physical examination. General physical examination included examination of metabolic, endocrine, cardiovascular, respiratory, gastro-intestinal and neurological systems to exclude presence of abnormalities.–

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

The HCC was diagnosed by ultrasonography and computer tomography (CT) as well as liver histopathological examinations. Other features including gender, age, tumor size, liver function tests, serum tumor marker alpha fetoprotein (AFP) level were done. The patients of HCC on top of viral hepatitis infection included in the study were positive for serum HCV RNA. Exclusion criteria were: diabetes mellitus, chronic renal failure, coronary artery disease, end stage liver disease, positive serum antinuclear antibody (ANA). Patients and controls had no history of other malignancy. All subjects were informed of the purpose of the study and informed consent was obtained. The study was approved by the ethical committee of Mansoura University.

Biochemical investigation:
Fasting blood samples (one ml) were collected on ethylenediamine tetraacetic acid (EDTA) and stored at –20°C for DNA extraction.

DNA extraction and investigation of IL-1 β and TNF-α polymorphisms:
Genomic DNA was extracted from peripheral blood using a Gentra genomic DNA purification kit (Qiagen; Hilden, Germany). The region containing SNP –31C/T within the IL-1β gene was detected by restriction fragment length polymorphism (RFLP) (Hwang et al., 2002). The forward primer used was: F: 5′-AGAAGCTTCCA CCAATACTC-3′. The reverse primer used was: R: 5′-GAAGCTTCCACCAATACTC-3′. Each PCR included 300 ng DNA, 200 mM dNTP, 500 nM primer, and 2.5 U Taq DNA polymerase (Ampli Taq Gold, Perkin-Elmer, Norwalk, CT, USA). DNA was initially denatured for one minute at 95°C, and then PCR amplification was performed in a PTC-200 thermal cycler (MJ Research, Essex, UK) using the following temperature program: 36 cycles of denaturation at 94°C for 45 seconds, annealing 54°C for 50 seconds, and extension at 72°C for 60 seconds. The PCR amplification was completed by a final extension at 72°C for 7 min. The amplification yielded a product of 448 bp. Upon cleavage with 5 U Alu I (Life Technologies Corporation. Invitrogen. Cat. no. 45200-029, China) overnight at 37°C, the DNA fragments were detected using 3% agarose gel stained with ethidium bromide and visualized under UV light. C allele: 344,79, 20 and 5 bp , while T allele: 247, 97,79, 20 and 5 bp (figure 1).

The region containing SNP IL-1β –511 C/T was detected by RFLP (Mansfield et al., 1994). The forward primer used was: F: 5′-TGGCATTTGATCTGGTTCATC-3′, The reverse primer used was: R: 5′-GTTTAGGAATCTTCCCACTT-3′. DNA was initially denatured for one minute at 94°C, and then PCR amplification was performed via 30 cycles of denaturation at 94°C for one minute, annealing 55°C for one minute, and extension at 72°C for one minute. The PCR amplification was completed by a final extension at 72°C for 7 min. The amplification yielded a product of 304 bp. Upon cleavage with 5 U Ava I (Gibco BRL, Life Technologies Corporation) overnight at 37°C, agarose gel electrophoresis revealed 3 patterns: normal genotype TT with 304 bp fragments, heterozygous mutated genotype CT with 304, 190, and 114 bp fragments, and homozygous mutated genotype CC with 190, and 114 bp fragments (figure 2).

Amplification of 410 bp for allele A1, 240 bp for allele 2 within intron 2 of IL-1RN VNTR using primers: Forward : 5′- TCCTGGTCTGCAGGTAA-3′ Reverse : 5′ CTCAGCAA CACTCCTAT-3′, initial 1 minutes denaturation at 96 °C followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60 for 1 min, extension at 70°C for 60 seconds and a final extension for 7 minutes at 70 °C (Wilkinson et al., 1999) (figure 3).

Amplification of an 836 base pair (bp) fragment of the TNF-α promoter region at position -308 (G-A) was amplified by amplification refractory mutation system (ARMS) (Sargen et al., 2000) using primers; a common forward primer 5′-CTGCATCCCCGTCTTTCTCC-3′ and one of two reverse primers with a 3′ mismatch corresponding to a G or and A at position –308. 5′-ATAGGTTTTGAGGGGCATCG-3′ specific for G or 5′-ATAGGT TTTGAGGGGCATCA-3′ specific for A. DNA was initially denatured at 96°C for 3 minutes, then 30 cycles of 96°C for 45 seconds, 55°C for 80 seconds, and 72°C for 2 minutes followed by 3 minutes extension at 72°C (figure 4).

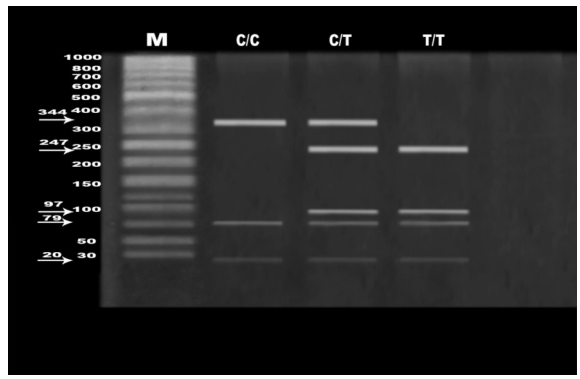

Figure (1): Ethidium bromide stained 2% agarose gel shows enzymatic digestion of – 31C/T polymorphism of IL-1β gene for different groups studied; Lane M: 50 bp DNA size marker, lane 1 (C/C genotype), lane 2 (C/T genotype) and lane 3 (T/T genotype).

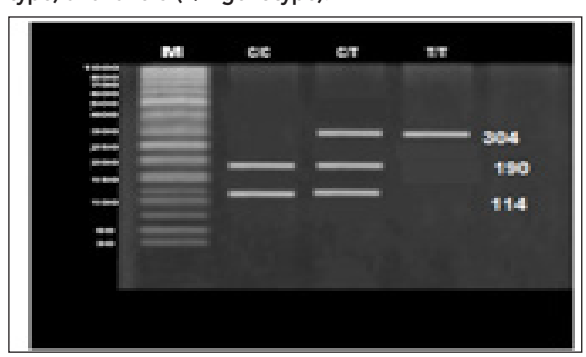

Figure (2): Ethidium bromide stained 2% agarose gel shows enzymatic digestion of -511C/T polymorphism of IL-1β gene for different groups studied; Lane M: 50 bp DNA size marker, lane 1 (C/C genotype), lane 2 (C/T genotype) and lane 3(T/T genotype).

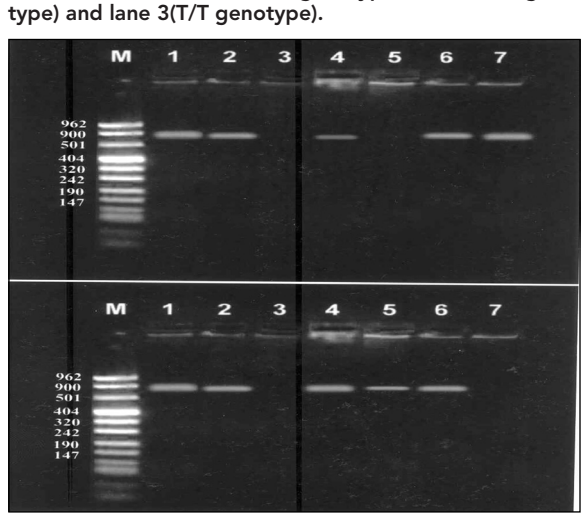

Figure (3): PCR amplification for TNF-α at position –308 (G-A) polymorphisms using SSP for G allele (above) and A allele (below) show: lane M, DNA size marker; lanes 1,2,4,6 (+ve for G and A alleles giving G/A genotype), lane 5 (-ve for G allele and +ve for A allele giving A/A genotype), lane 7 (+ve for G allele and -ve for A allele giving G/G genotype) and lane 3 –ve control. Band size: 836 bp.

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

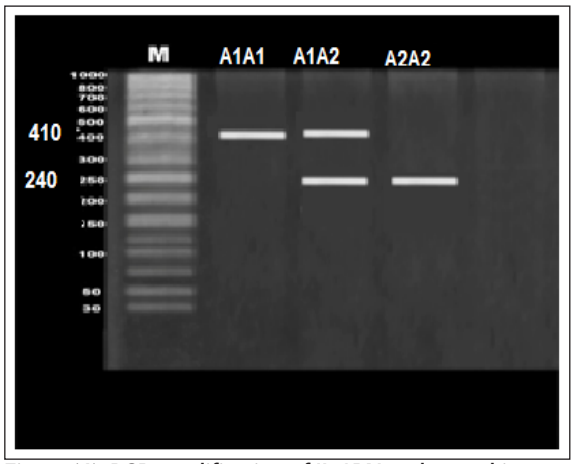

Figure (4): PCR amplification of IL-1RN polymorphisms using SSP for two alleles show band size 410 for A1 and 240 bp for A2

Statistical Analysis

The statistical analysis of data has been done by SPSS program statistical package for social science version 10. The description of the data done in form of mean ± standard deviation (SD) for quantitative data and frequency and proportion for qualitative data. The analysis of the data was done to test statistical significant difference between groups. For quantitative date student T-test was used to compare between 2 groups. One way ANOVA test was used to compare more than 2 groups. Chi square test was used to compare qualitative data. P is significant if <0.05 at confidence interval 95%.

Results:

Descriptive and biochemical data in Control group and HCV Patients with or without HCC are demonstrated in table (1). It is shown that there were significant increase in the serum level of ALT, AST and total bilirubin in both HCV groups. But, there was significant decrease in the serum albumin level in the same groups when compared to control group. Also, there was significant increase in the serum level of AFP in HCC group when compared to other two groups (all <0.05).

PCR determination of genotypes frequencies of IL-1β–31, IL-1β–511, IL-1RN VNTR and TNF-α–308 are given in table(2). It is shown that there were significant increase in the frequency of IL-1β–31 TT, IL-1RN A2A2 and TNF-α–308 AA genotypes in HCC group when compared to control . In addition, there was significant increase in IL-1RN A2A2 genotype frequency in HCV patients without HCC when compared to control group (<0.05). However , no significant difference in IL-1β–511 genotype frequencies between different groups.

Analysis of allele frequencies of IL-1β–31, IL-1β–511, IL-1RN VNTR and TNF-α–308 are given in table (2). It is shown that there were significant increase in the frequency of IL-1β–31T, IL-1RN A2 and TNF-α–308 A alleles in HCC group when compared to control (All p < 0.05, OR (95% CI) = 2(1.2-3.5), 4.3(2.4-7.8) and 2.3(1.7-6.4), respectively. In addition, there was significant increase in IL-1RN A2 allele frequency in HCV patients without HCC when compared to control group (< 0.001, OR (95% CI) =3.8(2.1-6.9). However, no significant difference in IL-1β–511 allele frequencies between different groups.

Table (1): Descriptive and biochemical data in Control and HCV groups :

Parameter	Control N= 70	HCV-patients with HCC N= 50	HCV-patients without HCC N= 50
Age (year) Mean ± SD	56.02±5.62	57.55±5.33	56.45± 4.56
Sex No (%) Male Female	35 (50%) 35 (50 %)	31 (62%) 19 (38%)	32 (64%) 18 (36%)
Tumor size (cm) Mean ± SD	NA	NA	2.04±0. 41
INR	1.11 ± 0.02	1.35 ±0.03	1.38 ±0.04
ALT (U/L)	19.36 ± 0.56	44.54 ± 2.89	57.35 ±3.58
AST(U/L)	18.36 ± 1.11	78.95 ± 5.44	87.08 ±5.33
Albumin (gm%)	4.22 ±0.09	2.86 ±0.16	2.66 ± 0.11
Total bilirubin (mg%)	0.84 ±0.06	1.80 ±0.17	1.84 ± 0.18
HCV-Ab	Negative	Positive	Positive
HCV-PCR	Negative	Positive	Positive
AFP µg/ml	9.45±5.7	256± 126.9	1828±1426.4

Abbreviations: ALT, alanine aminotransferase; AST, Aspartate transaminase; AFP, alpha-fetoprotein; INR, international normalized ratio; Ab, antibody; PCR, polymerase chain reaction, NA: not applicable.

Table (2):IL-1β–31, IL-1β–511, TNF-α–308and IL-1RN VNTR genotype frequencies among cases with hepatitis C virus compared to controls with their statistical significance

Genotypes	Control group (N=70)	HCV-patients with HCC (N=50)	HCV-patients without HCC (N=50)
IL-1β–31			
TT	19 (27.1 %)	21 (42 %)a	16(32 %)
TC	22 (31.4 %)	18 (36 %)	20(40%)
CC	29 (41.5 %)	11 (22 %) a	14 (28%)
IL-1β–511			
TT	29 (41.4 %)	20 (40 %)	18(36%)
TC	25 (35.7 %)	22 (44 %)	24(48%)
CC	16 (22.9%)	8 (16 %)	8 (16%)
TNF-α–308			
GG	56(80 %)	25 (50 %)a, b	35(70%)
GA	8 (11.4 %)	15 (30 %)a, b	8(16%)
AA	6 (8.6%)	10 (20 %) a	7 (14%)
Il-1RN			
A1A1	41 (59 %)	6 (12 %)a	6(12%)a
A1A2	26(37 %)	32 (64 %)a	35(70%)a
A2A2	3 (4%)	12 (24%)a	9 (18%) a

a: There is statistically significant difference when compared to control group

b: There is statistically significant difference when compared to HCV-patient group without HCC.

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

Table (3): IL-1β–31, IL-1β–511, TNF-α–308 and IL-1RN VNTR allele frequencies among cases with hepatitis C virus compared to controls with their statistical significance

Allele	Control group (N=70)	HCV-patients with HCC (N=50)	HCV-patients without HCC (N=50)	OR1 (95% CI) P1	OR2 (95% CI) P2	OR3 (95% CI) P3
IL-1β–31 T C	60 (43%) 80(57%)	60 (60%) 40(40%)	52 (52%) 48(48%)	2 (1.2-3.5)* 0.008	1.44(0.8-2.5) 0.2	1.4(0.8-2.4) 0.3
IL-1β– 511 T C	83(59.3%) 57(40.7%)	62 (62%) 38(38%)	56(56%) 44(44%)	1.1(0.7-1.9) 0.7	0.9(0.5-1.5) 0.6	1.3(0.7-2.3) 0.4
TNF-α– 308 G A	120(86%) 20(14%)	65 (65%) 35 (53%)	78(28%) 22(22%)	3.2(1.7-6.4)* <0.001	1.69(0.8-3.5) 0.2	1.9(1.02-3.6)* 0.04
IL-1RN A1 A2	108(77%) 32(23%)	44 (44%) 56(56%)	47(47%) 53(53%)	4.3(2.4-7.8)* <0.0001	3.8(2.1-6.9)* <0.0001	1.1(0.6-1.9) 0.7

OR1 between control &HCV with HCC
OR2 between control & HCV without HCC
OR3 between HCV with HCC & HCV without HCC
***= significant**

Discussion:
Hepatitis C virus (HCV) is a major cause of chronic liver disease (Shepard et al., 2005). Approximately 80% of infected patients fail to clear the virus and progress to chronic hepatitis. Some of those with chronic HCV infection may progress to liver cirrhosis and eventually hepatocellular carcinoma (HCC) (Lauer and Walker, 2001). Because risk for HCC increases with the severity of hepatic inflammation and chronic inflammation developing through the action of various inflammatory mediators is known as a cofactor of carcinogenesis (Coussens and Werb, 2002).

In this study, we examined the association of IL-1β –31 and –511 gene polymorphisms with development of HCC in patients with hepatitis C virus. It was found that TT genotype and T allele of IL-1β –31 but not IL-1β –511 were more frequent in HCC on top of HCV compared to control group. Those findings are in accordance with Wang et al. (2003) who stated that the proportion of IL-1β–31 TT genotype was higher than T/C and C/C genotypes in patients with HCC and concluded that IL-1β–31 genotype T/T is associated with presence of HCC in Japanese patients with chronic HCV infection. On other hand, Okamoto et al. (2010) reported that the IL-1β–31 genotype has no relationship with HCC diagnosis, but the higher transcriptional genotype, the –31T homozygote, has a poorer prognosis than C allele carriers among HCC patients and that gene polymorphism might be potential marker for predicting the prognosis of HCC patients.

The IL-1β–31 polymorphism, which involves TATA box, is reported to affect lipopolysaccharide induced IL1β production by modifying DNA protein interactions. It is hypothesized that the IL-1β–31 T allele enhances IL-1β production in the liver and induces hepatocyte damage that may finally lead to HCC development (El-Omar et al., 2001).The IL-1β–31 and –511 were in near complete linkage disequilibrium as was observed in previous reports(Takamatsu et al., 2000) indicating that one or both of these loci may functionally affect HCC development in patients with chronic HCV infection.

Our results found that A2A2 genotype and A2 allele of IL-1RN gene were more frequent in HCC on top of hepatitis C virus rather than control group. The IL-1RN gene has a pentaallelic 86-bp tandem repeat polymorphism (variable number tandem repeat; VNTR) in intron 2, of which the less common allele 2 (IL-1RN*2) is associated with a wide range of chronic inflammatory and autoimmune conditions. IL-1RN*2 is associated with enhanced IL-1β production in vitro (Santtila et al., 1998) and in vivo (Hwang et al., 2002).

In the current study, we found that AA genotype and A allele of TNF-α–308 gene were more frequent in HCC on top of hepatitis C virus rather than control group. In considering the relationship between –308 polymorphisms and the severity of HCV infection, Barrett et al. (2000) and Hohler et al. (1998) reported an association between these factors in patients with chronic HCV infection; Romero-Gomez et al., (2004), found no association between –308 polymorphisms and the severity of fibrosis in HCC. The –308A promoter variant has been associated with enhanced transcription of TNF-α (Wilson et al., 1997), but other investigations have failed to confirm this finding (Brinkman et al., 1996).Yee et al. (2000) found that –308Awas associated with increased cirrhosis in HCV infected patients. SNPs at –308 showed inconsistent associations with various HCV outcomes (Constantini et al., 2002). These discrepancies may be due to ethnic differences in the examined populations, leading to a differential distribution of cytokine gene polymorphisms.

Conclusion:
IL-1β , IL-1RN VNTR, and TNF-α genes polymorphism could be risk factors for the development of HCC in patients with chronic HCV in Egyptian population , and to confirm this results, large number of cases and other risk factors should be studied in the future.

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

REFERENCE Abbas, Z., Moatter, T., Hussainy, A. , et al. (2005). Effect of cytokine gene polymorphism on histological activity index, viral load and response to treatment in patients with chronic hepatitis C genotype 3. World J. Gastroenterol.; 11(42):6656-6661. | Akpolat, N., Yahsi, S., Godekmerdan, A., et al. (2005). Relationship between serum cytokine levels and histopathological changes of liver in patients with hepatitis B. World J. Gastroenterol.; 11(21): 3260-3263. | Barrett, S., Collins, M., Kenny, C., et al. (2003). Polymorphisms in tumor necrosis factor-alpha, transforming growth factor beta, interleukin-10 , interleukin-6, interferon-gamma, and outcome of hepatitis C virus infection. J. Med. Virol. 71(2): 212-218. | Bidwell, J., Keen, L., Gallagher, G., et al. (2001). Cytokine gene polymorphism in human disease: on line databases, supplement 1. Genes Immun, ; 2(2):61-70. | Bosch, F. X., Ribes, J., Cleries, R. & Díaz, M. (2005). Epidemiology of hepatocellular carcinoma. Clin. Liver Dis.; 9: 191-211. | Brinkman, B., Zuijdgeest, D., Kaijzel, E., et al. (1996). Relevance of the tumor necrosis factor alpha (TNF-α) -308 promoter polymorphism in TNF-α gene regulation. J. Inflamm.; 46(1):32-41. | Chang, Y. W., Jang, J.Y., Kim, N. H., et al. (2005). Interleukin-1α (IL-1 α) polymorphisms and gastric mucosal levels of IL-1α cytokine in Korean patients with gastric cancer. Int. J. Cancer; 114: 465-471. | Constantini, P., Wawrzynowicz-Syczewska, R., Clare, M., et al. (2002). Interleukin-1, interleukin-10 and tumour necrosis factor-alpha gene polymorphisms in hepatitis C virus infection: an investigation of the relationships with spontaneous viral clearance and response to alpha- interferon therapy. Liver; 22(5):404-412. | Coussens, L. M. & Werb, Z. (2002). Inflammation and cancer. Nature;420:860–867 | Craxi A. , Laffi G. & Zignego A. L. (2008). Hepatitis C virus (HCV) infection: A systemic disease. Molecular Aspects of Medicine ; 29, 85–95. | Cua, I., Hui, J., Bandara, P., et al. (2007). Insulin resistance and liver injury in hepatitis C is not associated with virus specific changes in adipocytokines. Hepatology; 46(1):66-73 | Dinarello, C. A.(1996). Biologic basis for interleukin-1 in disease. Blood; 87: 2095-2147. | El-Omar, E. M. , Carrington, M., Chow, W. H., et al. (2000). Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature; 404: 398-402. | El-Omar, E. M., Carrington, M., Chow, W. H., et al. (2001). Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature ;412:99. | Freedman, L. S., Edwards, B. K., & Ries, L. A. G. , eds. (2006). Cancer incidence in four member countries (Cyprus, Egypt, Israel, and Jordan) of the middle east cancer consortium (MECC) compared with US SEER. National Cancer Institute. Bethesda, MD: NIH Pub. No. 06-5873. | Hohler, T., Kruger, A., Gerken, G., et al. (1998). Tumor necrosis factor alpha promoter polymorphism at position -238 is associated with chronic active hepatitis C infection. J. Med. Virol.;54 (3):173-177. | Hwang, I. R., Kodama, T., Kikuchi, S., et al. (2002). Effect of interleukin-1 polymorphisms on gastric mucosal interleukin 1 beta production in Helicobacter pylori infection. Gastroenterology; 123: 1793- 1803. | Khattab, M. A., Eslam, M., Sharwae, M. A., & Hamdy, L. (2010). Seroprevalence of hepatitis C and B among blood donors in Egypt: Minya Governorate, 2000-2008. Am. J. Infect. Control; 38: 640-641. | Koziel, M. J. (1999). Cytokines in viral hepatitis. Semin. Liver Dis.; 19:157-169. | Lauer, G., &Walker, B. (2001). Hepatitis C virus infection. N. Engl .J. Med.; 345(1):41-52. | Levrero, M. (2006). Viral hepatitis and liver cancer: the case of hepatitis C. Oncogene; 25(27):3834-3847. | Mansfield, J. C., Holden, H., Tarlow, J. K., et al. (1994). Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. Gastroentrology; 106(3): 637-42 | Okamoto, K., Ishida, C.,, Ikebuchi Y., et al. (2010).The Genotypes of IL-1 beta and MMP-3 are Associated with the Prognosis of HCV-related Hepatocellular Carcinoma. Inter. Med.; 49: 887-895. | Romero-Gomez, M., Montes-Cano, M., Otero-Fernandez, M., et al. (2004). SLC11A1 promoter gene polymorphisms and fibrosis progression in chronic hepatitis C. Gut; 53(3):446-450. | Roshak, A. K., Jackson, J. R., McGough, K., et al. (1996). Manipulation of distinct NF kappaB proteins alters interleukin-1 beta-induced human rheumatoid synovial fibroblast prostaglandin E2 formation. J. Biol. Chem.; 271: 31496- 31501. | Sargen, K., Demaine, A. G. , & Kingsnrth, A.N. (2000). Cytokine gene polymorphism in acute pancreatitis. JOP;1(2)24-35. | Santtila, S., Savinainen, K., & Hurme, M. (1998). Presence of the IL-1RN allele 2 (IL1RN*2) is associated with enhanced IL-1b production in vitro. Scand J Immunol.; 1998; 47:195–8. | Shepard, C., Finelli, L., & Alter, M. J. (2005). Global epidemiology of hepatitis C virus infection. Lancet Infect Dis.; 5(9):558-567. | Takamatsu, M., Yamauchi, M., Maezawa, Y., et al. (2000). Genetic polymorphisms of interleukin-1α in association with the development of alcoholic liver disease in Japanese patients. Am. J. Gastroenterol;95:1305-1311. | Wang, Y., Kato, N., Hoshida, Y., et al (2003). Interleukin-1α gene polymorphisms associated with hepatocellular carcinoma in hepatitis C virus infection. Hepatology; 37: 65-71. | Wilkinson, R. J., Patel, P., Llewelyn, M., et al. (1999). Influence of polymorphism in the genes for the interleukin (IL)-1 receptor antagonist and IL-1beta on tuberculosis. J. Exp. Med.; Jun 21; 189(12): 1863–74. | Wilson, A., di Giovine,. F, Blakemore, A., et al. (1992). Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by Nco I restriction of PCR product. Hum. Mol. Gene; 1(5):353. | Yahia, M. (2011). Global health: A uniquely Egyptian epidemic. Nature; 474: S12–S13. | Yee, L., Tang, J., Herrera, J., et al. (2000). Tumor necrosis factor gene polymorphisms in patients with cirrhosis from chronic hepatitis C virus infection. Genes Immun.;1(6):386–390 | Zaltron, S., Spinetti, A., Biasi, L., et al. (2012). Chronic HCV infection: epidemiological and clinical relevance. BMC Infect. Dis.;12 (2). |