

Hepatitis C Virus genotypes in the Kingdom of Saudi Arabia

Abimbola O. Osoba, MD, FRCPath (UK).

ABSTRACT

Hepatitis C virus genotypes have been associated with specific geographical areas and in many cases with specific mode of transmission. In developed countries, genotype determination has formed a part of the management of patients with hepatitis C virus seropositivity and liver diseases due to hepatitis C virus. The epidemiology of hepatitis C virus has been shown to be changing rapidly in many countries due to population movement and different life-styles; hence the distribution of the genotypes is being monitored closely in many countries. In the Kingdom of Saudi Arabia, there are only a handful of publications recording the hepatitis C virus genotypes in various population groups. These studies have been carried out mainly in Riyadh (Central province) and Jeddah (Western province). There are no studies emanating from the Eastern or Northern provinces. According to these studies, the most prevalent genotype in the Western Province and probably in the whole Kingdom of Saudi Arabia was genotype 4, followed by genotypes 1a and 1b. Genotypes 1, 2a/2b, 3 and 6 are very rare in the Kingdom of Saudi Arabia. Genotype 5 was identified exclusively in the Western province and nowhere else. Genotypes 1b and 4 were associated with different histological grades of liver disease. Mixed infections with more than one genotype were observed in some studies. More detailed epidemiological studies of hepatitis C virus infections are needed in the Kingdom of Saudi Arabia to gain more insight into a possible type/subtype-specific pathogenesis of hepatitis C virus in the different regions of the Kingdom of Saudi Arabia as well as the distribution of the genotypes in the various localities.

Keywords: Hepatitis C virus, genotyping, epidemiology.

Saudi Med J 2002; Vol. 23 (1): 7-12

Chronic infection with Hepatitis C virus (HCV) is estimated to affect 170 million people worldwide and 20%-30% of these will eventually progress to liver cirrhosis and its sequelae, such as hepatocellular carcinoma.¹ In the Kingdom of Saudi Arabia (KSA) sero-prevalence studies suggest that the overall anti-HCV positivity is 0.99%, while in subjects over the age of 50 years it is 3.5%-5%.^{2,3} This prevalence rises to 75% among intravenous drug users in Jeddah.⁴ The non-parenteral route has been recognized as the major route of transmission of HCV in KSA.⁴ However this non-parenteral route has not been clearly elucidated. Most of the evidence to date suggests that intrafamilial and sexual transmission do not account for the majority of HCV transmission in KSA.⁵ Using logistic regression Poynard et al¹

identified 5 independent factors significantly associated with response to interferon therapy of HCV infection : genotypes 2 or 3; viral load less than 2 million copies/ml; age 40 years, minimal liver fibrosis stage and female gender. It is therefore important that the genotype of the HCV be determined prior to therapy, as it has implications for diagnosis, management and response to therapy. Moreover, HCV genotype determination assays have been particularly useful in studying the worldwide and local evolutions of the HCV endemics, since the epidemiology of HCV is changing rapidly.^{6,7} There are at least 11 genotypes and 90 subtypes of HCV and the prevalent HCV genotypes have been determined in most developed countries.^{6,8} There are few publications on HCV genotype determinations in

From the Molecular Biology Laboratory Division of Microbiology, King Khalid National Guard Hospital, Jeddah, Kingdom of Saudi Arabia.

Address correspondence and reprint request to: Dr. Abimbola O. Osoba, Consultant Microbiologist, Department of Pathology, King Khalid National Guard Hospital, PO Box 9515, Jeddah 21423, Kingdom of Saudi Arabia. Tel. +966 (2) 6240000. Fax. +966 (2) 6247444. E-mail: osobaa@ngha.med.sa

the KSA and these have indicated that the predominant genotypes were either type 1 or 4.⁹⁻¹⁷ The purpose of this communication is to review the publications on HCV genotyping in the KSA to ascertain the epidemiology of HCV in different parts of the KSA and to correlate the genotypes observed with liver histology among the various groups of HCV positive patients in KSA.

Biological characteristics of the HCV. The HCV genome is a positive-sense single stranded ribonucleic acid (RNA) genome of 9.4kb encoding one large polyprotein of 3010–3033 amino acids. The polyprotein is cleaved into structural proteins core, envelope 1(E1), and envelope 2 (E2) and several non-structural proteins (NS2 – NS5)^{6,18-20} (**Figure 1**). Different isolates from various parts of the world show considerable variation in their genomic sequence.²⁰ Based on these genomic differences, HCV has been classified on the basis of similarity of nucleotide sequence into 6 major genetic groups designated genotypes, 1–6^{6,18,19} and a series of more closely related subtypes designated a, b, c and are distributed worldwide. Recently, some blood donors in Vietnam could not be grouped into any of the common genotypes 1-6 and these isolates have been classified temporarily as genotypes, 7, 8 and 9.⁶ The genetic heterogeneity in HCV is found within individual strains of HCV and has been termed 'quasispecies'.¹⁸⁻²³ The quasispecies composition of HCV results from accumulation of mutations during viral replication in the patient. It has been speculated that the genetic heterogeneity has important pathobiological implications and may be responsible for the differences in liver progression and response to interferon therapy commonly seen in HCV infected patients.^{18,24} In addition, this biological characteristic of the HCV is believed to contribute to viral persistence in the presence of host immune system and during treatment with antiviral agents.²³

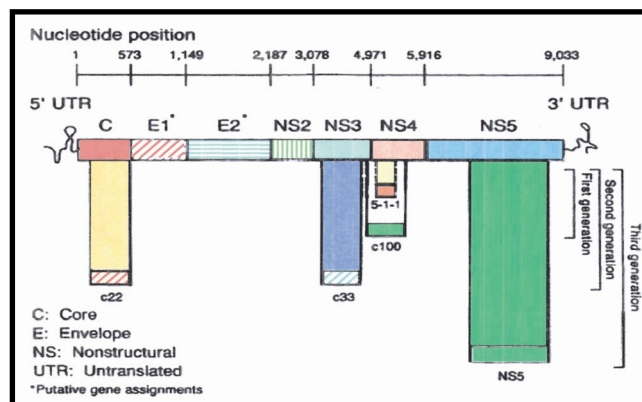


Figure 1 - Genomic organization of hepatitis C virus. First generation, 2nd generation and 3rd generation refer to serologic assays for detection of hepatitis C virus antibodies (Zein 2000¹⁸).

Genotyping methods. Various methods of typing HCV have been used to study isolates from different parts of the world. Genotyping techniques are based on detection of genotype-specific nucleotide sequences in the HCV genome. The reference method is sequence analysis of the genome followed by sequence alignments and phylogenetic analysis.^{18,19,25} Full-length sequence analysis is the most reliable method but it is labour intensive and expensive. However, analysis of more limited regions of the genome, HCV NS5, core, E1 and 5'UTR also allows for reliable classification.^{18,19} Presently, 3 rapid genotyping methods are available viz; (a) type-specific polymerase chain reaction (PCR) amplification with various sets of genotype-specific primers; (b) polymerase chain reaction amplification with conserved primers followed by restriction fragment length polymorphism (RFLP) analysis of PCR products with various sets of restriction enzymes and (c) polymerase chain reaction amplification of conserved primers followed by reverse hybridization to genotype-specific oligonucleotide probes coated on to microtitre plates or nitrocellulose strips.^{18,23-26} Based on the 3rd methodology, 3 commercial kits have been developed, viz: (i) hepatitis C virus Line Probe Assay (INNO-LIPA HCV, Innogenetics, Belgium); (ii) The Gen.Eti.K DEIA assay (Sorin Biomedics, Italy, and (iii) the Amplicis HCV assay (CisBio, France).^{6,8,18}

Prevalence of HCV genotypes worldwide. The differential prevalence of HCV genotypes appears to be linked to the geographic areas of origin, and the possible relationship between HCV genotypes and clinical expression of disease has recently aroused considerable interest.²⁷ The genotypes and their subtypes coexist in various geographic locations but show different prevalence and genetic diversity.^{6,28} Subtypes 1a and 1b are the most common genotypes in North America, followed by 2b and 3a. In the United Kingdom (UK), the most common genotype was genotype 1, followed by genotype 3, while intravenous drug abusers (IVDA) were more likely to have genotype 1a.²⁹ In Europe, the predominant genotypes are 1a, 1b, 2 and 3, while genotype 2c is found commonly in northern Italy, Argentina and Scotland.^{6,18} In some studies in the UK and Europe, it has been observed that genotype 1b is more common in patients who acquired HCV through blood transfusion,²⁹ while in Japan, subtype 1b is the most common subtype followed by subtypes 2a and 2b. However, in the Far East, HCV infection was due mainly to genotype 1 and in a lower frequency to genotypes 2, 3 and 6. Genotype 3a is particularly common among IVDA in Europe and USA, but rarely encountered among non-IVDA HCV infected individuals.^{6,18} It has been suggested that this genotype originated from the Indian subcontinent or South East Asia and was introduced into

Table 1 - Studies on hepatitis C virus genotyping in the Kingdom of Saudi Arabia.

Authors	N of samples & specimen types	N (%) of samples of Hepatitis C virus genotypes									
		1	1a	1b	2a/2b	3	4	5	6	U	Mixed genotypes
Saeed et al 1994	4 (BD)	0	0	3 (75)	0	0	1 (25)	0	0	0	0
El-Faleh et al 1995	28 (CH)	0	4 (14)	6 (21)	1 (4)	0	17 (61)	0	0	0	0
	32 (CRF)	0	8 (25)	7 (22)	7 (3)	0	16 (55)	0	0	0	0
Okamoto et al 1996	32 (H)	0	2 (6)	10 (31)	0	0	0	0	0	20 (63)	5 (4)
Al-Ahdal et al 1997	119 (CH)	0	12 (10)	20 (17)	3 (3)	2 (2)	57 (48)	0	0	20 (17)	2 (13)
Al-Ahdal et al 1998	15/23 (LT/S)	0	11 (73)	1 (7)	0	0	1 (7)	0	0	-	2 (20)
Ibrahim et al 1998	10 (CAH/NLH)	0	2 (20)	1 (10)	0	0	4 (40)	1 (10)	0	0	0
Fakeeh & Zaki 1999	37 (OP)	0	15 (41)	4 (11)	1 (3)	0	17 (46)	0	0	0	0
Shobokshi et al 1999	84 (CAH)	0	0	13 (14)	3 (4)	4 (5)	62 (74)	1 (1)	1 (1)	0	0
	39 (CRF)	1 (3)	13 (33)	4 (10)	0	0	19 (49)	5	0	0	0
	31 (IVDA)	0	3 (9)	12 (39)	1 (3)	3 (9)	11 (36)	1 (3)	0	0	1+
Osoba et al 2001	140 (CAH/NLH)	0	18 (13)	16 (11)	2 (1)	0	97 (69)	2 (1)	0	0	5 (4)*

N=number, U=unclassified/untypeable, M=mixed infection, H=hepatitis patients, NLH=normal liver histology, CRF=chronic renal failure + Hepatitis C virus liver disease, LT/S=liver tissues and Sera from Transplant patients, CAH=chronic active hepatitis, IVDA=intravenous drug abusers, BD=blood donors, CH=chronic hepatitis, OP=outpatients, *=5 patients with mixed infections (4&5), +=1 patient had mixtures of 1a, 1b and 4

industrialized countries probably through travellers.⁷ Genotype 4 seems to be confined to North Africa, especially Egypt and the Middle East,^{6,9,18,30} while genotype 5 has been found mainly in South Africa and genotype 6 in Hong Kong and Vietnam. Genotypes 7, 8 and 9 have been identified in only Vietnamese patients and genotypes 10 and 11 were observed in patients in Indonesia.¹⁸ Some authors have suggested that genotypes 7 to 11 are variants of the same group and should be classified as members of genotype 6.^{31,32}

Prevalence of HCV genotypes in the KSA. At least 9 studies have been carried out to determine the prevalence of HCV genotypes in various population groups in KSA.^{9-17,30} The prevalence of each genotype in the population groups studied has been summarized in **Table 1**. Most of these studies have been carried out in Institutions in the Riyadh area (Central Province). The patients studied included HCV sero-positive outpatients, blood donors, and patients with chronic hepatitis, liver cirrhosis, chronic renal failure and transplant patients. In 6 of these studies^{10,12,14-17} genotype 4 was recorded as the most common genotype, ranging from 40%-74%

among the various groups of patients studied (**Table 1**), which is the genotype reported to be most prevalent in the Middle East, Egypt and some African countries.^{6,33} Some studies however have reported different prevalent genotypes. Borisikin et al³⁰ genotyped 80 HCV isolates from KSA and found 6 (7.5%) 1b, 30 (37.5%) type 4 and 44 (55%) were type 3. They concluded that type 4 might not be the only dominant genotype in KSA. Similarly, Al Ahdal et al¹³ found the most prevalent genotype to be 1a in liver tissues and transplant patients (73%), while another 2 studies reported genotype 1b as the most common genotype among blood donors and hepatitis patients,^{9,11} although the number of samples examined was small. All the 4 studies carried out in the western region agree that the most prevalent genotype is type 4 ranging from 40%-74%.¹⁴⁻¹⁷ Although the most prevalent genotype in the central province appears to be genotype 4, the prevalent genotype in the Eastern and Northern provinces is yet to be ascertained. In most of the studies, the prevalence of the genotypes was the same between the sexes.^{10,17} Genotype 1b, which according to some authors is usually unresponsive to interferon, was

reported to be prevalent in 7%-31% of the populations studied in 8 of the studies reviewed here, while one study reported 3 out of 4 blood donors with genotype 1b.⁹ This genotype is highly prevalent in European countries.³ However studies from the UK and Europe suggest that genotype 1b, is frequently found among those who acquire the infection through blood transfusion. However, in the KSA the HCV seropositive patients with various grades of liver disease in the various studies may have acquired genotype 1b through blood transfusion, possibly before blood donors were screened for HCV. Interestingly, in the only study that examined IVDAs, the most prevalent genotype was found to be genotype 1b in 12 out of 31 (39%) of IVDAs,¹⁶ confirming the transmission by blood through infected syringes in this group of patients. This is in contrast to findings in Europe, where the most prevalent genotype among IVDAs was subtype 3a.⁶

Mixed infections were rarely encountered, but were observed at a prevalence rate of 4%-13% both in the Western and Central provinces.^{2,13,16,17} In one study in the Western province, mixed infections occurred in 5 patients (4%), and all were a combination of genotypes 4 and 5,¹⁷ compared to 4% reported in the Riyadh area, with mixed infections with genotypes 1a, 1b, 3a, 3b and 4,¹⁶ (Table 1). However, genotype 5 which has been identified mainly in South Africa^{6,18} has been rarely encountered in the KSA. It has been reported only in the Western province.^{14,16,17} It is unclear why this genotype has been found only in the Western province and whether it is present in the Eastern or Northern provinces. So far there is very little contact between South Africans and Saudis. It may have evolved independently in the KSA. Blood donors in European countries were almost exclusively infected with genotypes 1, 2 and 3, whilst 3a was found in a high percentage of intravenous drug users.^{6,29,34} Only one study in the KSA investigated the genotypes in HCV seropositive blood donors, but the number examined was too small to make any judgment on the most prevalent genotype in blood donors.⁹ Overall, in all the studies reported in the KSA, genotypes 1, 3, and 6 are very rare in the KSA. Only 2 studies in the KSA found genotype 3 in 2 (2%) of 119 patients¹² and 7 in (4.5%) 154 patients.¹⁶ Genotypes 1 and 6 were reported in only one study.¹⁶ Therefore in the management of the individual patients, it is desirable that genotyping be carried out not only to identify those patients with genotypes unlikely to respond to therapy but also those with mixed infections as this may impact on the management and prognosis.

Usefulness of genotyping. The main usefulness of molecular biology based genotyping techniques is direct access to viral genome sequence, high sensitivity due to amplification of targets by PCR and the possibility of determination of subtypes.^{6,7} The importance of HCV genotyping has considerably

increased in the last few years. It has been used to study the world wide and local molecular epidemiology of HCV and to trace sources of HCV infection in risk groups such as drug users and blood products. Typing has also been used to study relationships between type/subtype and the clinical status, pathogenesis and outcome of disease, or both.⁶ The major area of clinical application of HCV genotyping has been in the study of the significance of types/subtypes in response to antiviral treatment of HCV infection with Interferon and Ribavirin, as well as the identification of patients with mixed infections. It has also found a useful application in vaccine research and development.^{6,18,35}

One area of intensive research is the immunological aspect of HCV infection. Through genotyping, the genetic divergence of HCV in patients has been demonstrated. The quasispecies production in HCV has led to the selection of strains, which tend to avoid the immune system of the host.^{18,21-24} Cytotoxic T lymphocytes (CTL) are well known important components of protective immunity against viral infections. However, in HCV infection viral persistence occurs as the infection progresses due to its heterogeneity. The role of CTLs in protecting against viral persistence in HCV infection is presently unknown. Consequently, the development of an HCV vaccine would be difficult, but promising progress has been made.^{18,20}

Clinical importance of HCV genotypes. There is substantial evidence that HCV possesses different pathogenic potentials, since different responses to interferon treatment depending on the HCV type/subtype have been reported.^{6,27} Furthermore, association between some HCV types and the severity of liver disease has been noted in some reports.²⁷ Some of these have suggested that genotypes 2 and 3 are predictive of a favorable response to therapy than genotype 1.⁷ Bosmans et al³⁶ in a multicenter study genotyped HCV from 184 chronic hemodialysis patients and found that genotype 1b was the most prevalent genotype in Belgium, while in KSA the most prevalent genotype was 4 (36%), and types 1b and 1a (32% and 26%) were nearly as prevalent. Similar results were obtained in 2 studies carried out in the KSA.^{10,16} In these 2 studies, the most prevalent genotype among patients with chronic renal failure (CRF) was genotype 4, with prevalent rates ranging from 49%¹⁶ to 55%.¹⁰ The role of HCV genotyping as an epidemiological marker has been clearly substantiated. It has been used to trace HCV infections in risk groups such as IVDA, sources of infections in blood products and studies of worldwide molecular epidemiology. The issue of the pathogenicity of the different genotypes/subtypes remains controversial and long-term prospective studies in various population groups are still required.

HCV genotypes and liver disease. There is a considerable variation in the natural history of HCV infection in individual patients and progression to more severe liver pathology has been reported to depend not only on viral factors such as genotype, viral load, level of heterogeneity of HCV quasispecies but also on host factors such as age, sex, use of alcohol and length of time of HCV infection in the infected patient.^{1,18,23} There is very little consensus on disease cofactors and laboratory markers of progression of liver disease.³⁷ Opinion is divided among investigators as to the significance of genotypes in the evolution of severe liver disease. While some support the view of differential pathogenicity of the genotypes in progression of liver disease, others dispute the association.³⁷ Some investigators have produced strong evidence that among patients with chronic HCV seropositivity, infection with genotype 1b has been associated with a more severe liver disease and a more aggressive course than infections with other genotypes.^{18,38} In Japan where genotype 1b is more common than in Europe and USA, genotype 1b has been found to be more commonly associated with severe liver disease and hepatocellular carcinoma,^{18,39,40} than among HCV carriers in Western countries.^{41,42} In the UK, it has been reported that patients with genotype 1b had significantly more severe liver disease than other genotypes when the histological activity index was analyzed.²⁹ Some authors have refuted the association of genotypes with liver complications. It has been postulated that patients with genotype 1b tend to be older and therefore could have developed more severe liver disease due to chronicity of infections rather than genotype.^{43,44} However, on the other hand most investigators agree that genotype 2 pursues a biochemical silent clinical course.³⁴ One study in the KSA attempted to correlate genotypes and types with liver pathology and observed that 2 genotypes (1b and 4) were associated with various grades of liver disease, from normal liver histology to liver cirrhosis¹⁷ and that genotype 4 was associated with chronic active hepatitis in 95 (68%) of 140 subjects with HCV seropositivity. The severity of the histological lesions produced by each of the genotypes has not been ascertained in the KSA, but it appears that the most common genotype (genotype 4) produced a wide range of liver pathology.¹⁷ The issue of the pathogenicity of the different genotypes/subtypes remains controversial and long-term prospective studies in various population groups are required. Large series of data are required to determine the epidemiology of HCV genotypes and evidence-based strategy for the management of HCV liver diseases in the KSA. Our knowledge of the distribution of the HCV genotypes in the KSA is incomplete. More detailed epidemiological studies of HCV infections are needed in the KSA to gain

more insight into a possible type/subtype-specific pathogenesis of HCV in the different regions of the KSA. In countries where HCV genotypes have been carefully monitored, there is evidence that the epidemiology is changing rapidly and the distribution of the genotypes needs to be carefully monitored as they may be accompanied by changes in the clinical features of the disease and the subsequent response to therapy.⁷ Knowledge of the prevalence of asymptomatic HCV infection and prevalent genotypes has serious implications for the budget of healthcare providers, as treatment with interferon and other antiviral agents or liver transplantation is very expensive. Further research is needed to completely elucidate the epidemiology and pathogenesis of HCV infection, more so in KSA.

References

1. Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G et al. For the International Hepatitis Group (IHT). Randomised trial of interferon a2b plus ribavirin for 48 weeks or for 24 weeks versus interferon a2b plus placebo for 48 weeks for the treatment of chronic infection with hepatitis C virus. *Lancet* 1998; 352: 1426-1432.
2. Al Faleh FZ, Ramia S. Hepatitis V Virus (HCV) Infection in Saudi Arabia : A Review. *Annals of Saudi Medicine* 1997; 17: 77-82.
3. Bakir TMF. Age-Specific prevalence of antibody to Hepatitis C Virus (HCV) among Saudi population. *Saudi Med J* 1992; 13: 321-324.
4. Njoh J, Zimmo S. Prevalence of antibodies to Hepatitis C virus in drug-dependent patients in Jeddah. *East Afr Med J* 1997; 74: 89-91.
5. Arif M, Al-Swayeh M, Al-Faley, Ramia S. Risk of Hepatitis C virus among household contacts of Saudi patients with chronic liver disease. *J Viral Hepatitis* 1996; 3: 97-101.
6. Schreier E, Roggendorf M, Driesel G, Hohne M, Viazov S. Genotypes of Hepatitis C virus isolates from different parts of the world. *Arch Virol* 1996; 11 Suppl: 185-193.
7. Pawlotsky JM, Tsakiris L, Roudot-Thoraval F, Pellet C, Stuyver L, Dhumeaux D. Relationship between Hepatitis C virus genotypes and sources of infection in patients with chronic hepatitis C. *J Infect Dis* 1995; 171: 1607-1610.
8. Viazov S, Zibert A, Ramakrishnan K, Widell A, Cavicchini A, Schreier E et al. Typing of hepatitis C virus isolates by enzyme immunoassay. *J Virol Methods* 1994; 48: 81-92.
9. Saeed AA, Al Admawi AM, Rankin D, Olewicz G, McOmish F, Yap PL et al. Hepatitis C Virus RNA and Types in Saudi Blood Donors. *Saudi Med J* 1994; 15: 87-88.
10. Al-Faleh FZ, Huraib S, Sbeih F, Al-Karawi M, Al-Rasheed R, Al-Mofleh IA et al. Hepatitis C virus genotypes in patients with chronic liver disease and haemodialysis patients from Saudi Arabia. *J Viral Hepatitis* 1995; 2: 296-296.
11. Okamoto H, Kobata S, Tokita H, Inoue T, Woodfield GD, Holland PV et al. A second-generation method of genotyping hepatitis C virus by polymerase chain reaction with sense and antisense primers deduced from the core gene. *J Virol Methods* 1996; 57: 31-45.
12. Al Ahdal MN, Rezeig MA, Kessie G. Genotyping of Hepatitis C virus isolates from Saudi patients by analysis of sequence from PCR-amplified core region of the virus genome. *Annals of Saudi Medicine* 1997; 17: 601-604.
13. Al Ahdal MN, Kessie G, Rezieg MA, Ali MA, Nezamuddin N, Amin A et al. Hepatitis C virus detection and genotyping in liver tissues and sera of Saudi patients using PCR and a line probe assay. *Saudi Med J* 1998; 19: 182-184.

14. Ibrahim M, Osoba AO, Abdelaal MA, Al Shareef B. Hepatitis C virus detection and genotyping in liver tissues and sera of Saudi patients using PCR and a line probe assay. *Saudi Med J* 1998; 19: 640-641.
15. Fakeeh M, Zaki AM. Hepatitis C: Prevalence and common genotypes among ethnic groups in Jeddah, Saudi Arabia. *Am J Trop Med Hyg* 1999; 61: 889-892.
16. Shobokshi OA, Serebour FE, Skakni L, Al-Saffy YH, Ahdal MN. Hepatitis C genotypes and Subtypes in Saudi Arabia. *J Med Virol* 1999; 58: 44-48.
17. Osoba AO, Ibrahim M, Abdelaal MA, Al-Mowallad A, Al-Shareef B, Al-Haj HB. Hepatitis C Genotyping by Polymerase Chain Reaction and DNA Enzyme Immunoassay among Saudi patients in the Western Province, Saudi Arabia. *Annals of Saudi Medicine* 2000; 20: 394-397.
18. Zein NZ. Clinical significance of Hepatitis C Virus Genotypes. *Clin Microbiol Rev* 2000; 13: 223-235.
19. Simmonds P, Holmes TA, Cha TA, Chan SW, McOmish F, Irvine B et al. Classification of Hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J Gen Virol* 1993; 74: 2391-2399.
20. Choo QL, Richman KH, Han JH, Berger K, Lee C, Dong C et al. Genetic organization and diversity of the Hepatitis C virus. *Proc Natl Acad Sci* 1991; 88: 2451-2455.
21. Xavier F, Bukh J. Methods for determining the Hepatitis C genotype. *Viral Hepatitis Rev* 1998; 4: 1-19.
22. Zein NN, Persing DH. Hepatitis C genotypes : current trends and future implications. *Mayo Clin Proc* 1996; 71: 458-462.
23. Mizokami M, Ohno T. Determination of HCV Quasispecies by Cloning and Sequencing. In: Lau JY, editor. *Hepatitis C Protocols*, Totowa, (NJ): Humana Press Inc; 1998. p. 207-211.
24. Gonzales-Peralta RP. Detection of Hepatitis C Virus Quasispecies Heterogeneity by Single-Stranded Conformational Polymorphism. In: Lau JY, editor. *Hepatitis C Protocols*, Totowa, (NJ): Humana Press Inc. 1998. p. 213-220.
25. Simmonds P. Variability of Hepatitis C Virus. *Hepatology* 1995; 21: 570-583.
26. Davidson F, Simmonds P, Ferguson JC, Jarvis LM, Dow BC, Follet EAC et al. Survey of major genotypes and subtypes of Hepatitis C virus using RFLP of sequences amplified from the 5' non-coding region. *J Gen Virol* 1995; 76: 1197-1204.
27. Ravaggi AA, Zonaro A, Marin MG, Puoti M, Albertini A, Cariani E. Distribution of Viral Genotypes in Italy Determined by Hepatitis C Virus Typing by DNA Immunoassay. *J Clin Microbiol* 1994; 32: 2280-2284.
28. Vatteroni M, Maggi F, Morrica A, Fornai C, Giorgi M, Pistello M et al. Comparative evaluation of five rapid methods for identifying subtype 1b and 2c hepatitis C virus isolates. *J Virol Methods* 1997; 6: 187-194.
29. Watson JP, Brind AM, Chapman CE, Bates CL, Gould FK, Johnson SJ et al. Hepatitis C virus : Epidemiology and genotypes in north east of England. *Gut* 1996; 38: 269-276.
30. Boriskin YS, Bakir TM, Al-Aska AI, Booth JC. Is hepatitis C virus genotype 4 predominant in Saudi Arabia? *New Microbiologica* 1999; 22: 173-180.
31. Simmonds P, Mellor J, Sakuldamrongpanich T, Nucha Prayoon S, Tanprasert S, Holmes EC et al. Evolutionary analysis of variants of hepatitis C virus found in South East Asia: comparison with classification based upon sequence similarity. *J Gen Virol* 1996; 77: 3013-3024.
32. Tokita HH, Okamoto H, Iizuka H, Kishimoto J, Tsuda F, Miyakawa Y et al. The entire nucleotide sequences of three hepatitis C virus isolates in genetic groups 7-9 and comparison with those in the other eight genetic groups. *J Gen Virol* 1998; 79: 1847-1857.
33. Chamberlain RW, Adams N, Saeed AA, Simmonds P, Elliott RM. Complete nucleotide sequence of type 4 hepatitis C virus variant, the predominant genotype in the Middle East. *J Gen Virol* 1997; 78: 1341-1347.
34. Ravaggi AA, Mazza C, Puoti M, Marin MG, Cariani E. Hepatitis C Virus Genotypes in Northern Italy: Clinical and Virological Features. *Clin Microbiol* 1996; 34: 2822-2825.
35. Maertens G, Stuyver L. Genotypes and genetic variation of hepatitis C virus. In: TJ Harrison and Zuckerman AJ, editors. *The molecular medicine of viral hepatitis*. Chichester (UK): John Wiley & Sons Ltd; 1997. p. 182-233.
36. Bosmans JL, Nouwen EJ, Behets G, Gorteman K, Huraib SO, Shaheen FA et al. Prevalence and clinical expression of HCV genotypes in haemodialysis patients of two geographically remote countries: Belgium and Saudi Arabia. *Clin Nephrol* 1997; 47: 256-262.
37. Thomas DL, Astemborski J, Rai RM, Anania FA, Schaeffer M, Galai N et al. The Natural history of Hepatitis C Virus Infection, Host, Viral and Environmental Factors. *JAMA (Middle East)*. 2000; 284: 450-456.
38. Nousbaum JB, Pol S, Nalpas B, Landais P, Berthelot P, Brechot C and the Collaboratory Study Group. Hepatitis C virus type 1b (II) infection in France and Italy. *Ann Intern Med* 1995; 122: 161-168.
39. Takahashi M, Yamada G, Miyamoto R, Doi T, Endo H, Tsuji T. Natural course of chronic hepatitis C. *Am J Gastroenterol* 1993; 88: 240-243.
40. Yano M, Yatsunami H, Inoue O, Inokuchi K, Koga M. Epidemiology and long term prognosis of hepatitis C virus infection in Japan. *Gut* 1993; 34 Suppl 2: 13-16.
41. Di Bisceglie AM, Goodman ZD, Ishak KG, Hoofnagle JH, Melpolder JJ, Alter HJ. Long-term clinical and histopathological follow-up of chronic posttransfusion hepatitis. *Hepatology* 1991; 14: 969-974.
42. Hopf U, Moller B, Kuther D, Stemerowicz R, Lobeck H, Ludtke-Handjery A et al. Long-term of posttransfusion and sporadic chronic hepatitis non-A, non-B and frequency of circulating antibodies to hepatitis C virus (HCV). *J Hepatol* 1990; 10: 69-76.
43. Dusheiko G, Schmilovitz-Weiss H, Brown D, McOmish F, Yap PL, Sherlock S et al. Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. *Hepatology* 1994; 19: 13-18.
44. Pol S, Thiers V, Nousbaum JB, Legendre CH, Berthelot P, Krteis H et al. The changing relative prevalence of hepatitis C genotypes: evidence in hemodialyzed patients and kidney recipients. *Gastroenterology* 1995; 108: 581-583.