The Islamic University of Gaza
Deanery of Graduate Studies
Faculty of science
Medical Technology
Department

الجامعة الإسلامية – غزة
عمادة الدراسات العليا
كليـــــة العلـوم
قســـم التحاليل الطبية

Determination of Hepatitis C Virus Genotypes in Gaza Strip

By:

SOFIA SH. ZOUROB

Supervised By:

Dr. Basim M. Ayesh

Dr. Salah Abu- Jadallah

Submitted in partial fulfillment for the degree of Master of Science in Biological sciences- Medical Technology

June-2006

بِسْمِ اللّهِ الرَّحْمَنِ الرَّحِيمِ (1) الْحَمْدُ للّهِ رَبِّ الْعَالَمِينَ (2) الرَّحْمنِ الرَّحِيمِ (3) مالِكِ يَوْمِ الدِّينِ (4) إِيَّاكَ نَعْبُدُ وإِيَّاكَ نَسْتَعِينُ (5) اهدِنَا الصِّرَاطَ الْمُستَقِيمَ (6) صِرَاطَ الَّذِينَ أَنعَمتَ عَلَيهِمْ غَيرِ الْمَغضُوبِ عَلَيهِمْ وَلاَ الضَّالِّينَ (7)

صدق الله العظيم

سورة الفاتحة

DEDICATION

To

My parents----------

Brothers---------

Sisters----------

Friends----------

DECLARATION

I hereby declare that this submission is my own work and that to the best of my knowledge and belief, it contains no material previously published or written by another person nor material, which to a substantial extent, has been accepted for the award of any other degree of the university or other institute, except where due acknowledgment has been made in the text.

Signature----------

Sofia Sh. Zourob

1/07/2006

ACKNOWLEDGEMENT

My deepest and profound acknowledgments are due to my supervisor ***Dr. Basim M. Ayesh*** for his continuous support, generous help and fruitful and constructive suggestions.

I would like also to express my sincere thanks to the Islamic university of Gaza, **Dr Abood ELkishawe** and my supervisor **Dr. Salah Abu-Jadallah**.

My sincere thanks go to my uncle ***prof. Shehata Zourob*** and my brother ***Dr. Hamdy Zourob*** for the help and support they gave me.

My appreciations are extended to ***Dr. Mohammad Abu Shahla*** the director general of the European Gaza hospital and ***Mr. Rushdi Rusrus*** the director of the laboratory in European Gaza hospital.

My thanks are extended to all my colleagues in the laboratory in European Gaza hospital. Special thanks are extended to my colleagues ***Yehyea shoubair, Kayed Ahmad, Ahmad Tabash, Manal Abu Shawareb, Sameer Abu-Eid, Kamel Mahfouz and Mohmad El- Batta***

My special deep and sincere gratitude is due to my parents, brothers and sisters for their support, help, and encouragement.

My thanks are extended to all those not mentioned in person and who contributed in any way during this research. I wish all of them a long and prosperous life.

ABSTRACT

Hepatitis C virus is a heterogeneous virus with 6 major genotypes and more than 50 subtypes. The present work aims to determine the commonest HCV genotypes in chronic patients of Gaza strip, Palestine. This study highlights the prognostic potential of determining the HCV genotype for patients as an integral part of their diagnostic and therapeutic regimen, and thus may be helpful in tailoring the therapy for HCV patients based on their virus genotype. If taken in conjunction with other factors important in therapy HCV genotyping will reduce the expenses of treatment when the duration of therapy is accordingly optimized, and thus allowing a larger number of patients to be considered for the therapy. Genotyping of HCV may also be useful for identifying some important origins of transmission and risk factors of the virus in Gaza strip.

The chronic HCV patients participating in the study were interviewed, and a questionnaire was completed and serum samples were obtained from each of them. The samples were analyzed to justify the presence of HCV RNA by reverse transcriptase polymerase chain reaction (RT/PCR), and the PCR products of 92 positive samples were purified and submitted for sequencing and genotype determination. ALT level was determined for each sample.

The results of this study showed that genotype 1 and 4 and their subtypes 1a, 1b, 4a and 4c/d are the major common HCV genotypes in Gaza strip. Genotype 4 and its subtypes are the predominant ones. Genotype 1 and its subtypes collectively contributed to 28.3% of the cases, while genotype 4 and its subtypes collectively contributed to 64.1% of the cases. Mixed infection with the two genotypes was also seen among 7.6% of the cases. Travelling, surgery and blood transfusion in an endemic HCV area are the major roots of HCV transmission in Gaza strip although other routs exist. The results also show that genotype 1 infections are more serious than genotype 4 infections and associated with significantly higher ALT levels in patients' blood. The education level, infection date and hemodialysis don't show any significant relation to the HCV genotype and subtypes. The results of this study contribute to the identification of sources of outbreaks, risk factors and control strategy of HCV. The results will also help tailor treatment schedules as well as to monitor response to antiviral treatment.

ملخص

تحديد الطراز الجيني لالتهاب الكبد الفيروسي من نوع C في قطاع غزة.

يتميز التهاب الكبد الفيروسي من نوع C بكثرة التباين ونتيجة لذلك ظهرت له ستة طرز جينية رئيسة وأكثر من خمسين فرعية.

تهدف هذه الدراسة إلى تحديد الطراز الجيني لإلتهاب الكبد الفيروسي من نوع C في قطاع غزة، ومن ثم إلقاء الضوء على أهمية تحديد هذا الطراز باعتباره ركيزة أساسية في العلاج والتشخيص. كما انه يسلط الضوء على مصدر حالات التفشّي و يساعد في وضع إستراتيجية للحد من تفشي الفيروس. ولإنجاز ذلك أجريت دراسة ميدانية، استخدمت فيها إستبانه معدة خصيصاً لهذا الغرض، تم توزيعها على عينة من المرضى المصابين بهذا الفيروس في قطاع غزة والذين كانوا يترددون بانتظام على عيادات الباطنة الخارجية في مستشفى غزة الأوروبي بخانيونس أو مستشفى دار الشفاء بغزة بالإضافة إلى عيادة الرمال المركزية بغزة. ولقد تم جمع عينات دم منهم ومن ثم تحديد مستوى إنزيم (ALT) لكل عينة، كما وأجري لها فحص (PCR) للتأكد من وجود الحمض النووي للفيروس. و قد بلغ عدد العينات الموجبة إثنان وتسعين عينة تم تنقيتها وإرسالها لتحديد نوع الطراز.

أظهرت نتائج الدراسة أن هناك نوعان رئيسيان مختلفان من الطراز الجيني للفيروس من نوع C في قطاع غزة وهما: الطراز الأول من نوع 1 وما يندرج تحته ممن يسمى بتحت الطراز (1a, 1b) وكان يمثل ما نسبته 28.3% من أفراد العينة. أما الطراز الثاني فهو من نوع 4 وما يندرج تحته ممن يسمى بتحت الطراز (4a, 4c/d) وهذا يمثل ما نسبته 64.1% من أفراد العينة، مما يمكن القول بأن الطراز 4 هو السائد في قطاع غزة، هذا بالإضافة إلى وجود خليط ما بين الطرازين حيث بلغت نسبته 7.6% من أفراد العينة.

كما بينت نتائج الدراسة أن من الأسباب الرئيسة للإصابة بهذا الفيروس السفر إلى الخارج (ولاسيما عندما يكون السفر إلى مناطق تكثر فيها الإصابة) وعملية نقل الدم بالإضافة إلى إجراء العمليات الجراحية كما بينت النتائج أن هناك علاقة مابين المكان الذي تم التعرض فيه لهذه الأسباب والإصابة بأي من هذه الطرز، كما أظهرت النتائج أن هناك علاقة ذات دلالة إحصائية ما بين الطراز الجيني ومستوى إنزيم الكبد (ALT) في الدم الذي يعتبر من أهم وظائف الكبد الدالة على تحطم الخلايا الكبدية مما يدل على أن الإصابة بالطراز1 هي أكثر خطورة من الطراز4. بالمقابل لم تثبت الدراسة أن هناك علاقة ذات دلالة إحصائية مابين المستوى التعليمي أو تاريخ الإصابة أو عمليات غسيل الكلى.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

3' UTR	3' un translated region
5'UTR	5' un translated region
AIDS	Acquired Immunodeficiency Syndrome.
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
bDNA	branched DNA- technique
CDC	Center for Disease Control and Prevention
cDNA	complementary Deoxy ribonucleic acid
DNA	Deoxy ribonucleic acid
E1	Envelope glycoprotein 1 of hepatitis C virus.
E2	Envelope glycoprotein 2 of hepatitis C virus.
ELISA	Enzyme linked immunosorbant assay
FDA	Food and Drug Administration
GSP	Genotype specific primers
HCC	Hepatocellular carcinoma
HCV	Hepatitis C Virus
HVR1	Hypervariable region-1.
IFN	interferon
IRES	Internal ribosomal entry site.
LCR	ligase chain reaction
MOH	Ministry of health
NANB hepatitis	non-A, non-B hepatitis
NIH	National Institute of Health
NS	non structural protein.
ORF	Open reading frame**.**
PCR	polymerase chain reaction
Peg-IFN	pegylated interferon
RdRp	RNA-dependent RNA polymerase
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid

RT- PCR	Reverse transcriptase polymerase chain-reaction
SPSS	Statistical Package of Social Science.
SSCP	Single-strand conformational polymorphism.
SVR	Sustained virologic response
TMA	Transcription–mediated amplification
WHO	World Health Organization.

LIST OF TABLES

LIST OF FIGURS

INTRODUCTION

Overview

Hepatitis C is caused by infection of liver cells with the hepatitis C virus (HCV), which leads to severe inflammation of the liver with long term complications (1). HCV infections represent a serious medical problem and HCV is the major etiologic agent of sporadic and transfusion-associated non-A, non-B (NANB) hepatitis worldwide (2, 3). About 20%-30% of the newly infected persons show symptoms of acute hepatitis, while chronic infections develop in 70%-80% of adult infected persons and in 50%-60% of juveniles (4, 5).

The story of HCV began after the development of diagnostic tests for hepatitis A and hepatitis B viruses in the 1970s, when an additional parenterally transmitted agent, responsible for the majority of transfusion-associated NANB hepatitis cases was recognized (6). Since that time, radioimmunoassay (RIA), and later, enzyme linked immunosorbent assay (ELISA) techniques were developed for detection of antibodies produced against the major agent in patients with chronic NANB hepatitis. This agent has been given the name HCV (7). Since then HCV has become a focus of intensive research for several reasons. First, most HCV infections persist, leading to chronic hepatitis in the majority of cases, which can develop into chronic active hepatitis, liver cirrhosis and hepatocellular carcinoma (3). Second, HCV is distributed worldwide. According to the WHO, the number of people infected with HCV is continuously growing and has recently reached over 170 million worldwide (5). Third, the most effective HCV treatment is a high dose of pegylated interferon (Peg-IFN) plus ribavirin (8). This therapy is associated with a response rate of ~50% among patients infected with HCV genotype 1. Moreover the therapeutic regimen is expensive and is associated with a number of predictable toxicities, demonstrating the urgent need for more effective antiviral therapies (6, 9).

Worldwide HCV infections have reached epidemic proportions and became a major global health issue, with more than one million new cases reported

annually. The worldwide prevalence of chronic HCV infection is estimated to be 3%, varying from 0.5 to 5% in the general population of Western countries and USA while higher rates have been reported in some African and Southeast Asian countries (10, 11 and 12). Egypt, the closest neighbor to Gaza strip, has possibly the highest HCV prevalence in the world, where 10%-20% of the population are infected and HCV is the leading cause of hepatocellular carcinoma (HCC) and chronic liver disease in the country (13, 14). The high prevalence of HCV among Egyptian people may be due to the use of unsterile injection equipments during the mass treatment of the Egyptian population with antischistosomal therapy (13). Other neighbor countries such as Saudi Arabia, Syria and Jordan have HCV prevalence rates of 2.5%, 1% and 1.7% respectively (15, 16 and 17). In Israel, the reported prevalence of HCV infection is 0.5% (18).

According to the Palestinian ministry of health, surveillance of HCV in Palestine started in 1994, with an incidence rate of 2 HCV cases and carrier per 100,000 (19). The HCV incidence in Palestine was 4.2 and 5.8 per 100,000 in the years 2002 and 2003 respectively. In Gaza strip alone, 21,219 blood samples were screened in 2003 for Anti-HCV antibodies in the blood banks, among which 67 samples were found positive (prevalence rate of 0.3%). Moreover 19,164 samples from patients were tested for different reasons and 407 samples were found positive (prevalence rate of 4.4%) (19). The latest published HCV prevalence among blood donors, in 2004, was 0.2% (20). The calculation was done on 50,060 blood donors in Gaza strip and the West bank. In addition, 15,070 patients' blood samples were examined for different reasons with an HCV prevalence rate of 3.3% (20).

Studies suggest that over 170 million people around the world are infected with hepatitis C and are at risk of developing cirrhosis and liver cancer (5, 21). Currently, HCV is one of the main causes of liver-related morbidity and mortality, being responsible for an estimated 8,000 to 10,000 deaths annually in the world (4). Without large scale efforts to contain the spread of HCV and to treat infected

populations, the death rate from hepatitis C will surpass that of AIDS by the turn of the century and will only get worse (5).

After the complete HCV genome was determined, several HCV isolates from different parts of the world were obtained and sequenced. Comparison of the published sequences of HCV has led to the identification of several distinct types that may differ from each other by as much as 30% over the whole viral genome. Sequence variability is distributed almost equally throughout the viral genome, apart from the highly conserved 5′ UTR and core regions and the hypervariable envelope region (22).

The geographic distribution and diversity of HCV genotypes may provide clues about the historical origin of HCV. The presence of numerous subtypes of each HCV genotype in some regions of the world, such as Africa and Southeast Asia, may suggest that HCV has been endemic in these areas for a long time. Conversely, the limited diversity of subtypes observed in the United States and Europe could be related to the recent introduction of these viruses from areas of endemic infection (3). Evidence from molecular studies suggests that HCV subtype 1a and 1b are currently in a phase of epidemic growth that started ~100 years ago and the patients infected with these subtypes being older than those infected with other genotypes (23). A mixture of different genotypes is frequently seen in blood transfusion and hemodialysis patients as they are at higher risk of being repeatedly exposed to HCV infection (24, 25). HCV genotype has an effect on HCV replication in both individuals infected with HCV only and individuals co-infected with HIV-1 and HCV (26). There is increasing evidence that patients infected with different HCV genotypes may have different clinical profiles, severity of liver disease, and response to anti-HCV therapy (27). Moreover, HCV genotyping may shed light on its evolution, source of outbreaks, and risk factors. It has been used to identify the source of infection in cases of patient-to-patient transmission and is also useful in the study of other modes such as vertical (mother to baby), sexual transmission and needle stick injury (28, 29). Also HCV

genotype is essential for successful future research into vaccine development and control strategy (29).

Objectives

1. Determination of the different HCV genotypes distribution among Gaza strip patients who were previously diagnosed as HCV-positive patients by anti-HCV antibodies detection and/or RT-PCR.
2. Evaluation of some risk factors for HCV as potential contributors for HCV incidence in Gaza strip by analyzing the data obtained for HCV genotype and the patient personal information collected by a questionnaire.

Significance of this study

1. This study aims to highlight the prognostic potential of determining the HCV genotype for patients as an integral part of their diagnostic and therapeutic regimen.
2. The determination of the commonest HCV genotype in Gaza strip is helpful in tailoring the therapy for HCV patients based on their virus genotype.
3. If taken in conjunction with other factors important in therapy HCV genotyping will reduce the expenses for HCV patient's treatment when the duration of the therapy is optimized, and thus allowing a larger number of patients to be considered for the therapy.
4. Genotyping of HCV may be useful for identifying some of modes of transmission of the virus in Gaza strip.

LITERATURE REVIEW

Genome structure and organization

The genome of HCV is an enveloped single stranded positive-sense RNA virus with 9646 ribonucleotides, (Gene bank accession number: NC_004102, Gene bank ID: 22129792) and a poly-A tail at its 3'-end (10). Morphologically, HCV particles are spherical particles, measuring 55 to 65 (nm) with fine spike-like surface projections (Fig. 1) and its genome is organized in a fashion similar to that of the flaviviruses and pestiviruses (30, 31 and 32). It was lately classified in its own subgenera, Hepacivirus (21, 33 and 34). The HCV genome starts with a highly conserved untranslated region in its 5'-side (5' UTR: nucleotide 1-341) followed by a single open-reading frame (ORF: nucleotides 342-9377) that encodes for a polyprotein of about 3011 amino acids (Fig. 2). The ORF length characteristically varies according to the HCV genotype (3, 7, and10). In its 3' end HCV genome has another untranslated region (3' UTR) of about 27 bases which contains an upstream variable region (VR) within which substantial sequence diversity is evident between different genotypes of HCV (33). On the other hand, the highly conserved 5' UTR region shows up to 85% nucleotide sequence identity among different HCV isolates. Therefore it is used as a universal diagnostic locus for RT-PCR detection of HCV genome from all isolates. In addition it is used for genotype determination of the isolate based on the sequence variation in certain nucleotides of this region among the different genotypes. Moreover, the 5' UTR folds into a complex structure comprising an internal ribosome entry site (IRES) that is responsible for cap-independent initiation of the viral polyprotein translation (2, 31 and 33).

Post-translational processing of the polyprotein gives rise to three structural N-terminals HCV proteins (C, E1, and E2/NS2) and four C-terminal none-structural proteins (NS2, NS3, NS4, and NS5) that are believed to function in viral replication (35).

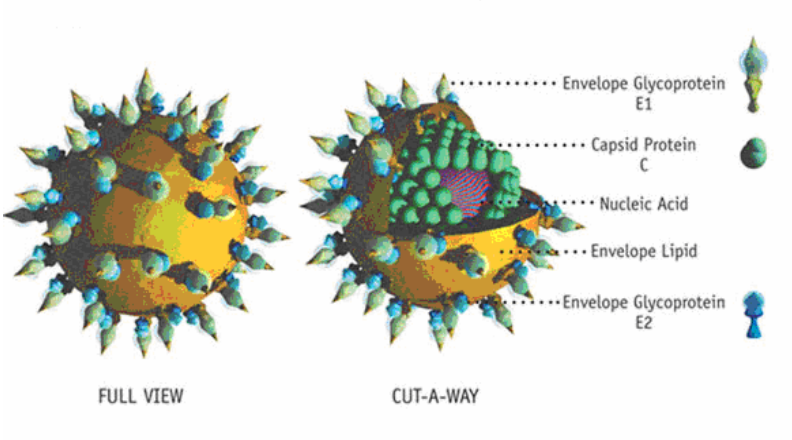

Fig. 1: A model of the human HCV particle shape and the main structural elements of its composition.

The Core protein (C) is located at the amino terminus of the polyprotein, measuring 30 to 35 nm in diameter and is highly basic in nature and considered likely to be the viral core (capsid) protein (11, 30). It is released from the viral polyprotein by nascent proteolytic cleavage at the amino acid 191 by cellular protease. The HCV core gene contains the most conserved sequence in the coding region of most HCV genotypes, which implies an important biological function (30). It interacts with several cellular proteins closely linked to important signal transmission pathway and can specifically suppress apoptotic cell death in artificial systems (11). Immunologically, (C) presents several epitopes for both T and B cells (30, 11). Thus it may have an immunomodulatory function and critical role in the establishment of persistence and disease pathogenesis (21).

E1 and E2, the major viral structural envelop glycoproteins, are released from the viral polyprotein by the action of host-cell signal peptidases (21). The E1 and E2 regions of the HCV genome show the highest mutation rate (31). E2 contains a Hypervariable region 1 which shows an extraordinarily high rate of nucleotides change, and frequently results in codon changes (31). The presence of this rapidly changing region may permit a mechanism by which HCV evades host immune response and establishes and maintains a persistent infection (3). The HCV envelop protein, E2, contains a sequence identical to phosphorylation

sites of the interferon (IFN) inducible protein kinase (PKR) and the eukaryotic translation initiation factor (eIF2), a target of PKR (2,11). Thus it blocks the interferon signaling in response to the HCV infection and confers resistance to interferon therapy in most HCV isolates (36).

The nonstructural proteins (NS2, NS3, NS4A, NS5A, and NS5B) respectively are autocleaved cysteine protease, serine protease/helicase, cofactor of NS3, PKR interacting protein, and RNA-dependent RNA polymerase (11). The polypeptides spanning NS3 to NS5B in the polyprotein are required for replication of the viral RNA, and most likely all contribute to a membrane-bound, macromolecular replicase complex. The NS5B protein is a highly conserved RNA dependent RNA polymerase (RdRp), which forms the catalytic core of this complex (21). It is capable of primer-independent initiation of RNA synthesis on an RNA template (33, 34).

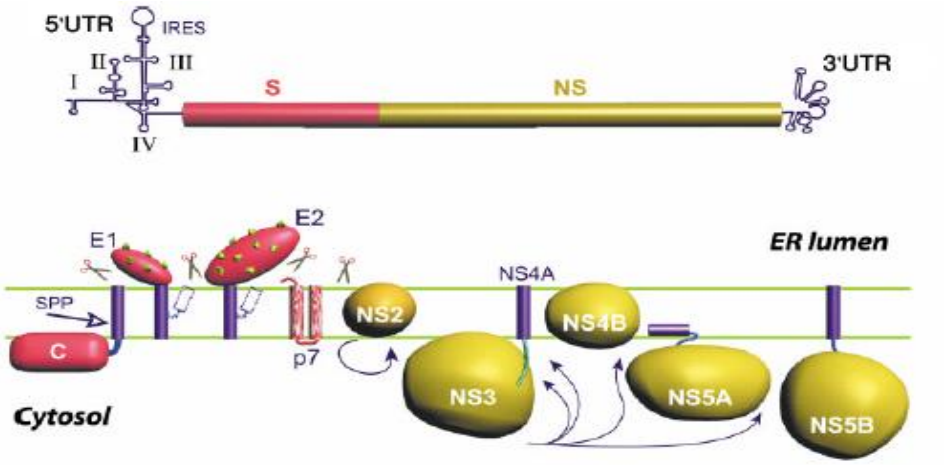

Fig. 2**: **Genomic organization and post translational processing of HCV in the endoplasmic reticulum (34).

Risk factors of HCV transmission

A person infected with HCV serves as a source of its transmission to others, and is at high risk for chronic liver disease and other HCV related chronic systemic disorder (37). Moreover patients with immunologic deficits are at an increased risk of developing chronic HCV infection (37).

HCV is efficiently transmitted by direct precutaneous exposure to infectious blood, sexual transmission, intravenous drug use, needle stick accidents, and blood transfusion before hepatitis screening (2, 31). Also transmission of the virus from mother to child is most commonly seen at the time of delivery, and is more frequent, with 3%–7.8% rate of transmission, in the case of high maternal viral load (38). Moreover, nosocomial transmission of HCV is frequently recorded (39, 40). Because most of the chronic HCV infections are generally anicteric and asymptomatic, patient-to-patient transmission of HCV during surgical procedures may occur (31, 37and 41). This may be due to contaminated anesthetics breathing circuits, inadequate cleaning of surgical equipment and faulty administration of intravenous anesthetic drugs (41). Other nosocomial transmission can occur during dental procedures, and hemodialysis (31).

Less common risk factors for the transmission of HCV are tattooing, ear piercing, body piercing or acupuncture with nonsterile equipments. The sharing of toothbrushes and razors is also a risk factor. There is no definite evidence however that HCV is transmitted by breast feeding, although some risk may exist if the mother has a high viral load (37).

In Gaza strip according to an unpublished study conducted by Mr. Rushdie Rusrus, the main risk factors for HCV infection are traveling abroad, especially to endemic areas; blood transfusion and nonsterile injections. Also visiting dentist for treatment, having surgery, and tattooing have important role in increasing the chance of HCV infection.

HCV genotypes

HCV is known to have a marked genetic heterogeneity with an estimated nucleotide substitution rate of between 1.44×10^{-3} and 1.92×10^{-3} substitutions per site per year (27, 34). The main source of HCV genetic variability is point mutations, single nucleotide substitutions, deletions or insertions (5). These mutations are introduced to the viral genome during its replication by the viral encoded RdRp. This process is highly error-prone because of the lack of proofreading activity of the RdRp (5, 34).

The accumulation of nucleotide substitutions in the HCV genome has resulted in its diversification and the evolution of the different HCV genotypes. At least six major genotypes of HCV and more than 50 subtypes have been identified (42). They differ by as much as 30%-50% of their nucleotide sequences (7). Each HCV genotype is given an Arabic number from 1 to 6, and the subtypes classified under each genotype are designated by an alphabetic letter (a, b, c, etc.) (43). Although HCV genotypes 1, 2, and 3 appear to have a worldwide distribution, the prevalence of the different genotypes varies by geographic region, see Table 1 (3).

Table 1: The geographic distribution of HCV genotypes in the world (14, 44 and 45).

Genotype	Most common geographic localization
1a	Mostly found in North & South America; also common in Australia
1b	Mostly found in Europe and Asia
2a	The most common genotype 2 in Japan and China
2b	The most common genotype 2 in USA and Northern Europe
2c	The most common genotype 2 in Western and Southern Europe
3a-f	Mostly found in Scotland & other parts of the United Kingdom; also common in USA
4a-f	Have been found in Egypt, Saudi Arabia, Jordan, Lebanon, Kuwait and most of the Eastern Mediterranean countries
5	Has been described in South Africa
6	In South-East Asia

The role of HCV genotypes in the progression of liver disease is one of the most controversial areas of HCV research, but it cannot be separated from other factors such as, viral load, alcohol intake, and length of time of HCV infection (3).

There is evidence that associates certain genotypes of HCV with more severe hepatic pathology or quicker progression to chronicity (46). For example genotypes 1b and 4 were found to be associated with different histological grades of liver disease. The rate of progression to chronicity after acute exposure to HCV was 92% in patients exposed to HCV genotypes 1b and 4, compared with 33%-50% in patients exposed to other genotypes (3, 47). Furthermore, genotype 1b was present in most of the patients with HCV-associated hepatocellular carcinoma and decompensate liver disease requiring liver transplantation, more than in those with chronic active hepatitis C (46, 47and 48). Also HCV genotype 4 patients have a worse prognosis compared to other HCV genotypes after liver transplantation (14). Moreover, patients infected with type 1 and 4 had a higher viral load than patients infected with other genotypes (46). On the other hand, HCV genotype 3 infections are more likely to resolve spontaneously than other genotypes, but may show faster progression of liver-fibrosis (23, 49). Studies also showed that patients with genotype 3 were less likely to have detectable HCV in their blood compared with genotype 1 patients, suggesting that the immune system had cleared the virus on its own (22). The majority of patients infected with genotype 3 still developed chronic hepatitis C, but this is significantly lower than the rate of chronic disease in patients with genotype 1 (22) .

Hepatocellular steatosis, a frequent histological feature of chronic HCV infection, has distinct mechanisms in different HCV genotype infections. Indeed it appears to be principally associated with metabolic factors in genotype 1 infection, whereas it is virus-induced in genotype 3 infection, and thus more severe in HCV genotype 3 than in genotype 1 infection (50).

HCV therapy and its relation to HCV genotype

There is no effective vaccine against HCV infection up to date, as the efforts to develop one are complicated by the extensive genetic and antigenic diversity among HCV strains, the absence of a robust immunity after natural infection, and the lack of tissue culture systems and small animal models (51).

When left untreated, however HCV can lead to serious complications that include cirrhosis, primary liver cancer, liver failure and death (1). So the goal of treatment is to prevent these complications, which is principally achieved by eradication of HCV infection (43, 52 and 53). The viral RNA structures and proteins play a critical role in the HCV life cycle, and thus represent ideal targets for specific HCV therapy (54).

In this regard there is no standard therapy for acute HCV infection and patients with symptomatic acute hepatitis C may not need immediate antiviral therapy (55). A recent study suggests that a follow up of at least 30 days is required to identify those patients who should receive treatment (56).

In chronic HCV infection, treatment with a high dose of interferon (IFN) for 12 months may be helpful (8, 57). Interferon was discovered in 1957 and subsequently named because of its ability to interfere in the life cycle of viruses (58). IFNs may be produced by cells in response to a range of stimuli, including foreign nucleic acids, foreign cells and bacterial and viral antigens (59). The recently FDA-approved pegylated interferon (Peg-IFN) is a modification of interferon, showing a decreased renal clearance, altered metabolism, and increased half life (52).

IFN or Peg-IFN therapy is usually recommended for patients with chronic HCV who are at greatest risk for progression to cirrhosis (35). These persons include anti-HCV-positive patients with persistently elevated ALT levels, detectable HCV RNA, and a liver biopsy that indicates either portal or bridging fibrosis or at least moderate degrees of inflammation and necrosis (35, 53). Other recommendations for whom therapy is widely accepted, include persons with compensated liver disease (total serum bilirubin <1.5 mg/dL, International Normalized Ratio (INR) < 1.5, Albumin > 3.4 g/dL; platelet count > 75,000 mm3; and no evidence of hepatic encephalopathy or ascites), and with acceptable hematological and biochemical indices (hemoglobin >13 g/dL for men and >12 g/dL for women, creatinine < 1.5 mg/dL) (52).

Several factors identifiable before therapy are independent predictors of response to either form of IFN. These include the baseline level of serum HCV and the HCV genotype (58, 59). Analysis of the changes in HCV titers in the early part of IFN treatment is useful for prediction of the therapeutic response (53, 58). HCV genotype is the single most important factor predicting treatment outcome, and hence has a strong influence on the choice of treatment strategy (53, 60).

During the first 1 to 2 days of therapy, and after a delay of 7-10 hours of IFN administration, the amount of viral RNA declines rapidly and then declines more slowly (58). In patients with genotype 1, the viral load decline in the early phase of IFN treatment is slower than that in patients with genotype 2 and 3, which may explain in part their lower rate of sustained virological response (SVR) (58). SVR is defined as the absence of the HCV RNA in serum by a sensitive test at the end of treatment and six months later (52).

Patients, who have relapsed after interferon monotherapy, can be treated with a combination of interferon and ribavirin for 6 months, if there are no contraindications to ribavirin (8, 61, and 62). Ribavirin (1-β -D-ribofuranosyl-1, 2, 4-triazole-3-carboxyamide) is a purine nucleoside analogue with broad spectrum antiviral activity against various RNA and DNA viruses (63). Given alone, ribavirin has not proved to be efficient in chronic HCV infection, but it significantly enhances the sustained viral clearance rate when combined to IFN (56). The NIH Consensus Statement on Management of Hepatitis C: 2002 summarizes that the SVR rate is low in patients with genotype 1 infection, with higher HCV RNA levels or with more advanced stages of fibrosis (1). In these cases a combination therapy might be useful (58). Research has shown that people with genotypes 2, 3 and 5 have a higher SVR rate to such therapy than genotype 1 and 4 (46, 48 and 60). Moreover patients with genotype 2 or 3 can be cured in almost 90% of cases by combination therapy with peg-IFN and ribavirin (55). On the other hand, genotype 1 and 4 infections require therapy for 48 weeks, whereas shorter treatment is feasible in genotype 2 and 3 infections (8, 64).

IFN alpha therapy is expensive, carries a substantial morbidity and has side effects such as neutropenia, thrombocytopenia, depression, hypothyroidism and hyperthyroidism, irritability, concentration and memory disturbances, visual disturbances, fatigue, muscle aches, headaches, nausea and vomiting, skin irritation, low-grade fever, weight loss, insomnia, hearing loss, tinnitus, interstitial fibrosis and hair thinning (31, 52 and 65). Ribavirin also usually causes serious side effects such as hemolytic anemia, which can be life-threatening for patients with ischemic heart disease or cerebral vascular disease, fatigue, itching, rash, sinusitis, birth defects, or gout and is teratogenic and therefore contraindicated during pregnancy (28, 31). The therapy also can cause complications to patients with preexisting anemia, bone marrow suppression, or renal failure (31, 65). Growth factors, such as epoetin and granulocyte colony-stimulating factor have been used to counteract the adverse events of ribavirin and interferon (52).

HCV therapy is usually very lengthy and expensive with 6 to18 months of treatment costing between $12,000 and $15,000 per patient (46). In Gaza strip this treatment costs between $ 9,000 and $18,000 (66).

HCV Genotypes in the Gaza Strip:

Currently, there is almost no available data about the most prevalent genotypes in the Palestinian territories. Moreover, genotyping of the HCV is not requested by Palestinian physicians as a part of the disease assessment protocol.

A study conducted in 1998 showed that the most common genotype found in Gaza strip is type 4 (67). The results of the study suggested that HCV root of transmission to Gaza strip is mainly from Egypt. This study however is not enough because it was carried out only on a small sample of a limited population of the Gaza strip (Nasser hospital patients). Moreover, the proportions of the disease have changed dramatically since that date.

Laboratory testing

The determination of HCV RNA level and genotype is an important step in the diagnosis of HCV infection and the patient's candidacy for therapy (68).

Screening and initial diagnosis of HCV is usually accomplished by ELISA detection of antibodies against HCV in the patient's blood. However, false-positive results may be obtained after the infection has resolved as the antibody titer remains high, and in individuals with hypergammaglobulinemia, as a result of autoimmune hepatitis (69). On the other hand, the incubation period of HCV infection varies from 6 to 12 weeks (average 7 weeks) (40) and false negative results are obtained in the first 3-4 months window-period needed for the infected person to mount detectable levels of anti-HCV antibodies (70). Such a false-negative result may be obtained in immunocompromised patients as well. To avoid these disadvantages, several molecular diagnostic methods have been developed that target the HCV ribonucleic acid (RNA) in the serum of suspected patients (71). These include the reverse transcriptase polymerase chain-reaction (RT/PCR); the signal amplification nucleic acid probe assay, alternatively known as branched DNA (bDNA); ligase chain reaction (LCR); transcription–mediated amplification (TMA) and other various post amplification hybrid capture systems. However, the RT/PCR technique has evolved as the most common method for HCV viral RNA detection and quantification in clinical samples (31). The direct detection of HCV RNA by RT-PCR allows the early diagnosis of acute infections (within 1-3 weeks of exposure) as well as the diagnosis of infection in immunosuppressed patients (10, 38). There are two challenges of molecular assays for HCV: (a) the RNA is unstable and the sample should be frozen if not assayed immediately, and (b) the virus is not always present in the bloodstream in an infected person. Negative RT-PCR assays for HCV are usually repeated three times over a three month interval before a definite diagnosis is made (43).

The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity is usually used to assess hepatocellular damage as a result of

HCV infection (72). However, elevation in serum aminotransferases activity is not HCV-specific, as it is seen in other liver disorders of various etiologies as well.

Qualitative HCV RT-PCR:

A highly sensitive and specific qualitative HCV RT-PCR test can tell whether the virus is actively replicating at the time of analysis. HCV-RNA qualitative PCR assays can detect as few as 50 copies of the viral RNA/ml serum when the primers are specific for the 5' UTR, the most highly conserved region among HCV genotypes (31).

Quantitative HCV RT-PCR:

The viral load detected with quantitative molecular assays can be used to predict the outcome of anti-HCV therapy but not the likelihood of disease progression (10). Currently there are some FDA-cleared commercially available techniques for quantifying the HCV RNA in serum. These include the "VERSANT® HCV RNA 3.0 Assay (bDNA)" from Bayer Diagnostics, and the "COBAS AMPLICOR HCV MONITOR® Test, v2.0" from Roche (52).

HCV genotyping

There are several commercially available methods for the determination of the HCV different genotypes. Some of these methods are FDA-cleared, such as "The VERSANT® Genotype Assay (LiPA)" from Bayer Diagnostics. This technique uses the reverse hybridization and detection of the HCV RT-PCR amplification products to multiple type-specific DNA probes immobilized on a single strip (73). Other techniques for HCV genotyping use the real time multiplex PCR technology to specifically detect the genotype-specific sequences by fluorescently labeled probes or primers (73). Researchers have also used genotype specific primers (GSP) in a multiplex PCR amplification that generates variable length products corresponding to the different HCV genotypes (27). The restriction fragment length polymorphism (RFLP) is widely used for HCV genotyping (74). However, sequencing of the PCR products generated from the 5' UTR of the HCV genome and the subsequent sequence analysis remains the golden technique for genotyping (31, 75).

MATERIALS AND METHODS

Study design

The current study is a retrospective study concerned with genotyping of HCV isolated from chronic patients in Gaza strip. The study design is based on data collected from each patient by dichotomous and multiple-choice based questionnaire and experimental procedure performed on serum samples. The collection of blood samples started in June 2006.

Study population

The study population was made up of chronic HCV Gaza patients who presented continuously to the outpatient medical clinics of the European Gaza hospital, Al-Shiffa hospital and the central laboratory in Al-Remal clinic. Male and female patients, with no age restriction, were chosen according to availability and based on the fact that they are chronically infected with HCV. The patients were almost equally distributed in the north and south of Gaza strip.

Setting

The setting of the study was the molecular biology laboratory in the Islamic university of Gaza.

Ethical considerations

The study principles were discussed and revised by the academic supervisors. The study was approved officially by the Islamic university, the Palestinian MOH, and Helsinki committee. The principle of the research and possible outcomes were explained to all subjects of the study and the samples and data were collected upon their agreement. The subjects' personal information was dealt with in complete confidentiality.

Questionnaire and data collection

A close-ended, dichotomous and multiple-choice based questionnaire was designed in Arabic and completed by the researcher to prevent any misunderstanding. The patients investigated in the study were interviewed before

completion of the questionnaire. The questions were direct and brief and included personal data like, age, marital status, education level and previous history of HCV infection. Refer to appendix (1) for the complete questionnaire.

Reagents

The reagents that were used are listed in table 2.

Table 2: Reagents which were used

Chemicals and reagents	**Manufacturer**
QIAamp Viral RNA Mini Kit: • Buffer of(AVL, AW1,AW2,&AVE) • Carrier RNA (poly A)	Qiagen, Germany
QIAGEN One Step RT-PCR Kit: • **One step RT-PCR enzyme mix:** 20mM Tris.Cl, 100mM KCl, 1mM dithiothreitol (DTT), 0.1mM EDTA, 0.5% (v/v) Nonidet p-40, 0.5% (v/v) Tween20, 50% glycerol (v/v) and stabilizer. The pH 9.0 at 20ºC. • **5x QIAGEN one step RT-PCR buffer**: the same balanced combination of potassium chloride (KCl) and ammonium sulphate (NH4)2SO4, Tris Cl, 12.5 mM MgCl2, DTT; PH 8.7. • **DNTP Mix**: 10mM of each of dATP, dCTP, dGTP and dTTP. • **RNase inhibitor**. 40 units/ µl. • **RNase-free water**.	Qiagen, Germany
Qiaquick Gel Extraction Kit:	Qiagen, Germany

Chemicals and reagents	Manufacturer
• Buffer PB contains guanidine hydrochloride and isopropanol • Buffer PN contains sodium perchlorate and isopropanol • Buffer QG contains guanidine thiocyanate • Buffer EB (10mM Tris.Cl, PH 8.5).	
Serum alanine aminotransferase test reagent	Diaysis
Ethyle alcohol 96%	Local
Isopropanol	Local
Agarose gel	Promega (USA)
50 bp DNA ladder	New England Bio Labs (USA
Low molecular weight marker	New England Bio Labs (USA)

Primers sequences

The primers listed in table 3 were designed to specifically bind the 5'UTR of the HCV genome that is highly conserved among the different genotypes (76). Sequence degeneracy was included to allow annealing to the various genotypes (1, 2 and 31).

Table 3: Primers sequences which were used.

Primer ID	Sequence (5' TO 3')*	Location	Product size (bp)
HCV-1F	CCCTGTGAGGAACT***W***CTGTCTTCACGC	-299	298
HCV-1R	GGTGCACGGTCTACGAGACCT	-1	

** To include sequence degeneracy, **W** = A or T.*

Equipment

The important equipments that were used are listed in table 4:

Table 4: The important equipments that were used.

Instruments	Manufacturer
Thermal-cycler: mastercycler gradient	Eppendrof, Germany
Electrophoresis chambers and power supply	Biorad, USA
Microcentrifuge	Sanyo, UK
UV-transilluminator	Hoefer ,USA
Microwave oven	L.G Korea

Methods

Patients and Samples collection

One hundred peripheral blood samples were collected, in plain tubes, from Gazian patients (males and females) who are chronically infected with HCV (based on previous diagnosis either by ELISA or RT-PCR). The samples were collected upon Helsinki committee approval, starting from June, 2005. Out of the 100 samples, 55 were obtained from *the European Gaza hospital* (south of Gaza strip), and 45 from *Al-Shiffa hospital* and *Al Remal clinic* (north of Gaza strip). Fifteen samples were collected from dialysis patients, and the rest from random patients. Serum samples were prepared within no more than 4 hours after sample collection and immediately stored in sterile DNase- and RNase-free, tightly caped tubes, at -70ºC.

Biochemistry

ALT was analyzed for all samples using (Alcyon autoanalyser machine) in the laboratory of the European Gaza hospital, according to the manufacturer instructions. The normal range for ALT was considered from 0 to 40 IU /ml.

Viral RNA Extraction

The viral RNA was extracted from serum samples using the QIAamp viral RNA Extraction kit according to the manufactures recommendations. Briefly, 140 µl from each sample were first brought to room temperature before being lysed under highly denaturing conditions, to inactivate RNases and to keep viral RNA integrity. The lyses buffer contains carrier RNA to increase the yield. The viral RNA was extracted by 96% ethanol and subsequently loaded onto the QIAamp spin columns that are designed to bind the RNA selectively. The columns were washed by ethanol-containing buffers and the RNA was eluted in a special RNase-free buffer. The RNA samples were either immediately processed for PCR or stored at –20ºC for later PCR Experiments.

HCV RT- PCR amplification

Both cDNA synthesis and PCR amplification of the target sequences were performed in a single tube using the QIAGEN one step RT-PCR kit according to the manufacture instructions. This allows for enrichment of the target sequence and increases the specificity using a sequence specific primer for cDNA synthesis and amplification. Moreover this would reduce the loss of sample and reduce the possibilities of contamination.

The reactions were carried out in 25µl reaction volumes using 10µl RNA in the presence of 0.6µM of each primer (HCV-1F and HCV-1R), 400µM of each dNTP and 5 units RNase inhibitor. The reaction cycling conditions were: 1 cycle at 50ºC for 30 minutes, 1 cycle at 95ºC for 15 minutes followed by 40 cycles of 95ºC for one minute, 55ºC for one minute and 72ºC for one minute. Finally the reactions were allowed to complete at 72ºC for 10 minutes and held at 4ºC.

The products were analyzed by running onto 2% agarose gel and stained with ethedium bromide. The appearance of a 298 bp band was considered a positive result. In each reaction set, a water sample was used as a negative control to exclude possible contamination.

PCR Band Gel Extraction

DNA fragments were purified from the gel using the *Qiaquick Gel Extraction Kit* according to the manufacture instructions. Briefly, the agarose gel which contains the DNA fragment was cut excised and dissolved by adding 3 volumes of the QG buffer to 1 volume of agarose gel. It was then incubated at 50ºC for 10 minutes. The PCR products were then extracted by the addition of isopropanol and loading onto the QIAquick spin columns that are designed to bind the DNA selectively. The columns were washed and the DNA was eluted in a special buffer that contains no EDTA. The DNA samples were labeled by a reference number and stored at –20ºC until submission for sequencing.

HCV genotyping and sequence analysis

Ninety two purified PCR products were submitted to (hy–laboratories Ltd, Park Tamar, Rehovot) for sequencing and genotypes determination. The sequencing procedure was conducted using the Big Dye Terminator Cycle Sequencing kit from ABI in an automated sequencer. The sequences were obtained as data files in two extensions for each file (*.ab1 and *.seq).

The genotypes of the different samples were determined by the hy–laboratories company using ABI-analysis software for downstream analysis of sequences. We also used the NCBI Genotyping internet software (77) for the same purpose. The sequence files were edited if necessary by the BioEdit Sequence Alignment Editor software freely available from (78) seeFig.3. The edited sequences were used to determine the genotype by the upper mentioned website. The edited sequences were also aligned and phylogenetic trees were constructed using the Internet version of the (ClustaW) multiple sequence alignment program for DNA or protein, using default parameters for DNA multiple alignment (79). The resulted files of alignment and trees were downloaded on the local disc and later manipulated using the Jalview version 2.07 java multiple alignment editors. The phylogenetic trees were constructed using the Unweighted Pair Group Method using arithmetic average (UPGMA). This method is a simple method that is used for constructing phylogenetic trees especially if

the rates of evolution are approximately constant among the different lineages tested. It involves the clustering of sequences by tiding a non-member sequence with the lowest average dissimilarity over cluster members.

Fig. 3: A sample for sequence viewed by the BioEdit software.

Data analysis

The questioner results were transformed into numerical scoring system, and the scorers were entered in the data sheets of the SPSS statistical data analysis software. The statistical analysis was carried out (by SPSS and Epi-info), using frequencies and cross tabulation between dependant and independent variables. Chi square test and T test were used and P-values of < 0.05 were considered statistically significant.

RESULTS

Amplification and sequencing of the RT-PCR products

All of the patients included in this study were chosen on the basis of being chronically infected with HCV as determined by either anti HCV test or a previous RT- PCR test. All patients were residents in Gaza strip. The setting of the study was molecular biology laboratory. Islamic university of Gaza.

RNA was extracted from serum specimens of each patient, and the RNA was then subjected to both reverse transcription of a complementary strand and to PCR amplification in a single test tube (see Materials and Methods). A fraction of the samples tested were found to be negative while 92 samples gave the 298 bp band on the gel characteristic of a positive PCR (Fig.4). The negative samples may be due to that the infection has resolved as the majority of patients are under therapy. These samples thus were excluded from the study as being irrelevant to the genotyping study. The quality of the PCR reaction and the lack of contamination were assessed by the inclusion of a negative (H2O), control which didn't show any PCR amplification in all of the trials.

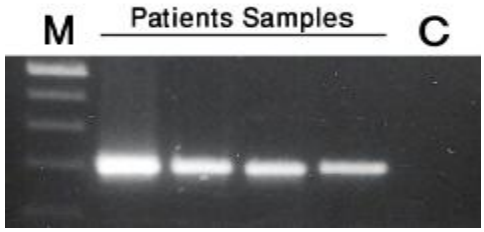

Fig. 4: The result of a representative PCR experiment of patients' samples with different degrees of viremia*. The level of viremia was previously determined by quantitative PCR performed in Al Remal Clinic.* ***M****: 100bp ladder, molecular weight marker;* ***C****: water control. The size of product is 298 bp.*

The detected HCV genotypes and subtypes in Gaza Strip

The major genotypes determined using the NCBI online service were in general agreement with those got from the hy–laboratories company. However

the sequences got from the hy–laboratories company included a wider range of subtypes and thus they were considered in the study.

Table 5 shows the distribution of the different HCV genotypes and their subtypes among 92 chronic HCV patients in Gaza strip. Our results indicate that there are only two major genotypes of HCV in Gaza Strip: Genotype 1 and its subtypes 1a and 1b collectively contributed to 28.3% of the cases (n=26), and genotype 4 and its subtypes 4a and 4c/d collectively contributed to 64.1% of the cases (n=59). Mixed infection with the two genotypes were also seen among 7.6% of the cases (n=7) (Fig. 5). The combined infections included genotypes 1 and 4 or 1 and 4c/d.

Table 5**: **Frequencies of HCV genotypes in the 92 patients studied in Gaza Strip.

Genotype	No of cases	Percent
Genotype 1 and its subtypes	**26**	**28.3**
1	1	1.1
1a	17	18.5
1b	8	8.7
Genotype 4 and its subtypes	**59**	**64.1**
4	19	20.6
4a	21	22.9
4c/d	19	20.6
Mixed Genotypes	**7**	**7.6**
1+4	2	2.2
1+4c/d	5	5.4
Total	**92**	**100**

Because of the small number of patients classified under most of the different subtypes, we collectively combined each genotype and all of its subtypes under the same category. This combination is considered for all of the statistical analysis in the rest of the study.

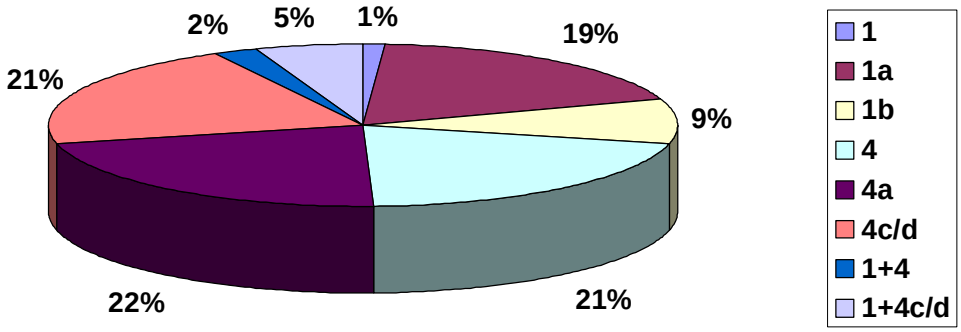

Fig. 5: Distribution of HCV genotypes and subtypes among the study population.

Relationship between HCV genotypes and the age of patient

The study population was subdivided into three age groups (0-39 years, 40-59 years and over 60 years). Table 6 shows the distribution of HCV genotypes among the three groups. The distribution of the genotypes and their subtypes in the entire population of the study is preserved among each of the age groups separately. Genotype 4 and its subtypes for example is the major among all of the three age groups as well as in the entire population (table 6).

We can hardly detect any significant relation between the genotypes and the three groups. However it is noteworthy to mention that infections by mixed genotypes are significantly more frequent in elder patients (over 60 years) (71.4%) when compared to genotype 1 for example (15.4%) (P-value = 0.01, odds ratio = 13.75) (Table 6 and Fig. 6).

Table 6: Distribution of the different HCV genotypes and subtypes among the age groups of the studied patients.

		Genotype			Total
		1, 1a, 1b	4, 4a, 4c/d	4+1, 4c/d+1	
0-39	*Count*	8	18	1	27
	% within age group	29.6	66.7	3.7	100.0
	% within genotype	30.8	30.5.	14.3	28.6
40-59	*Count*	14	25	1	40
	% within age group	35.0	62.5	2.5	100.0
	% within genotype	53.8	42.4	14.3	43.5
Over 60	*Count*	4	16	5	25
	% within age group	16.0	64.0	20.0	100.0
	% within genotype	15.4	27.1	71.4	27.2
Total	*Count*	26	59	7	92
	% within age group	28.3	64.1	7.6	100.0
	% within genotype	100.0	100.0	100.0	100.0

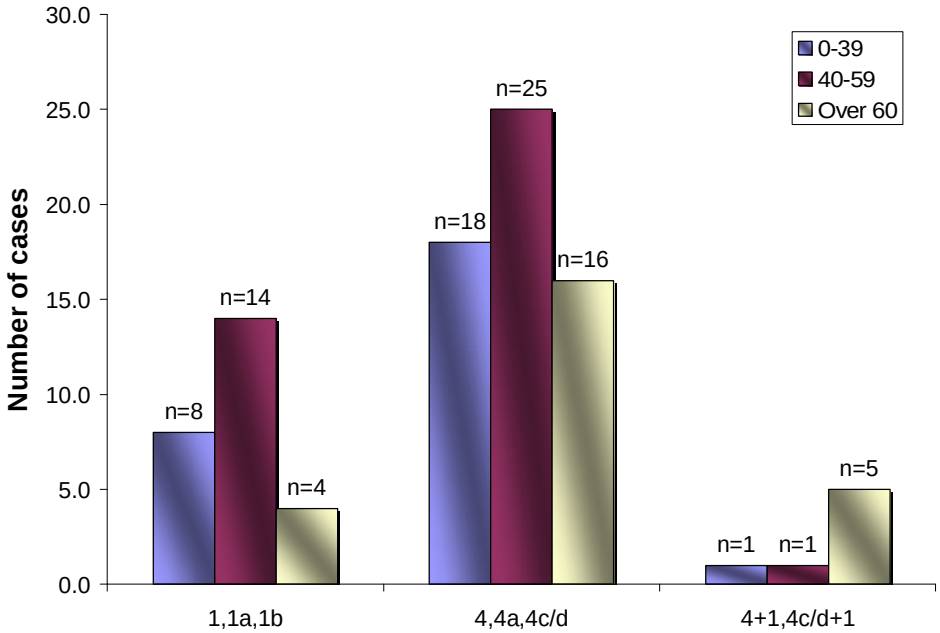

Fig. 6: Relationship between the HCV genotype and the age of patients. *The age of patients participating in the study (n =92) were subdivided into 3 age group (0-39, 40-59, and over 60) and the number of patients falling in each group was plotted for each genotype.*

Relationship between HCV genotypes and the patients place of living.

Fifty eight out of the 92 patients studied (63%) were from the south of Gaza Strip and 34 (37%) were from the north (table 7). The table shows that genotype 4 and its subtypes in the south (72.4%) are more prevalent than genotype 1 and its subtypes (19.0%). In contrast, genotype 4 and its subtypes contribute to (50.0%) of the Northern patients compared to (44.1%) for genotype 1 and its subtypes. The mixed genotypes are the least abundant among patients from both areas (8.6 % for Southern patients and 5.9% for Northern patients).

If we look at the distribution of genotype 4 and its subtypes only, we find that 71.2% of patients infected with this genotype are located in the Southern part of the Gaza Strip compared to 28.8% in the Northern part. This difference is statistically significant (P-value = 0.01; odds ratio=3.29). On the other hand,

genotype 1 and its subtypes are almost equally distributed among Southern patients (42.3%) and Northern patients (57.7%) (Fig. 7).

Table 7: Distribution of the different HCV genotypes and subtypes in the southern and northern parts of Gaza strip.

		Genotype			Total
		1, 1a, 1b	**4, 4a, 4c/d**	**4+1, 4c/d+1**	
South	***Count***	11	42	5	58
	% within "South"	19.0	72.4	8.6	100.0
	% within genotype	42.3	71.2	71.4	63.0
North	***Count***	15	17	2	34
	% within "North"	44.1	50.0	5.9	100.0
	% within genotype	57.7	28.8	28.6	37.0
Total	***Count***	26	59	7	92
	% within "Total"	28.3	64.1	7.6	100.0
	% within genotype	100.0	100.0	100.0	100.0

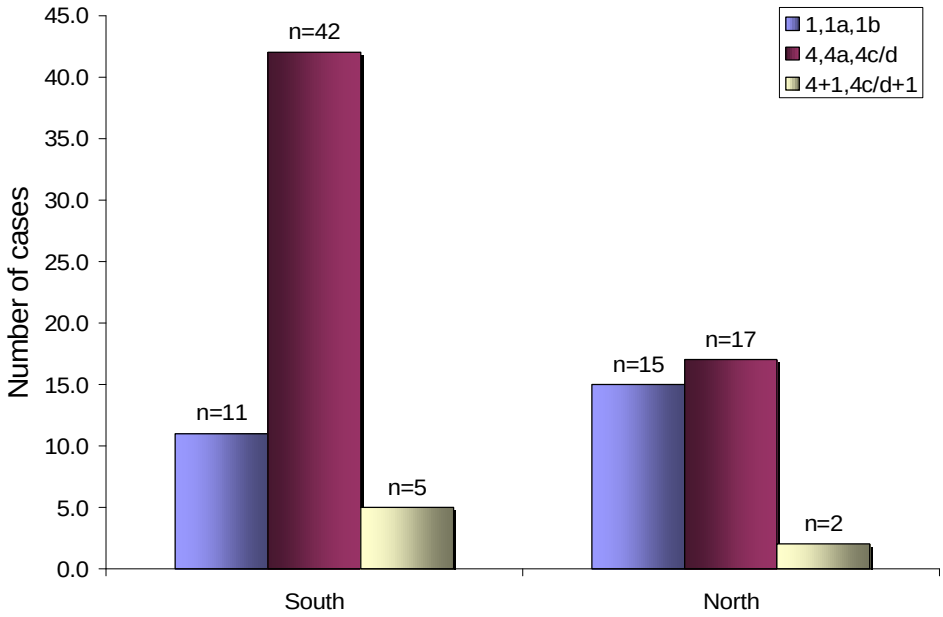

Fig. 7: Relationship between HCV genotypes and the patients place of living. *The patients studied were divided into two groups according to their place of living (X-axis). The distribution of HCV genotypes among each group is shown in the bar chart. The number of cases is indicated on the top of each bar.*

Relationship between HCV genotypes and the patient traveling history

Table 8 shows the distribution of HCV genotypes in relation to the patients traveling history. Collectively the number of patients that have traveled to a foreign country is almost twice that of those who haven't (62.0% n=57) compared to (38.0% n=35). Moreover, it is likely that traveling has contributed more to genotype 4 and its subtypes (62.7% "Yes") compared to (37.3% "No") and to the mixed genotypes (85.7% "yes") compared to 14.3% "No"). However no such contribution was made in the case of genotype 1 and its subtypes (53.8 %" yes") compared to 46.2% "No")**.**

***Table 8: Distribution of the different HCV genotypes and subtypes among patients who did or didn't travel to a foreign country*.**

		Genotype			Total
		1, 1a, 1b	4, 4a, 4c/d	4+1, 4c/d+1	
Yes	*Count*	14	37	6	57
	% within "Yes"	24.6	64.9	10.5	100.0
	% within genotype	53.8	62.7	85.7	62.0
No	*Count*	12	22	1	35
	% within "No"	34.3	62.9	2.9	100.0
	% within genotype	46.2	37.3	14.3	38.0
Total	*Count*	26	59	7	92
	% within "Total"	28.3	64.1	7.6	100.0
	% within genotype	100.0	100.0	100.0	100.0

Among the 57 patients that traveled abroad, 39 patients (68.4%) traveled before they were infected (Table 9). Only one patient (1.8%) reported that he traveled after he has been infected, while 17 patients (29.8%) reported that they don't know if they traveled before or after the infection (table 9). More or less, the same distribution is seen among each of the genotypes (50% compared to 7.1% for genotypes 1; 75.7% compared to 0% for genotypes 4 and 66.7% compared to 0% for the mixed infections). These percentages differences would reach greater proportions if we neglect patients who don't know.

Table 9: Relationship between genotype and traveling date.

		Genotype			Total
		1, 1a, 1b	**4, 4a, 4c/d**	**4+1, 4c/d+1**	
Before	***Count***	7	28	4	39
	% within "Before"	17.9	71.8	10.3	100.0
	% within genotype	50.0	75.7	66.7	68.4
After	***Count***	1	0	0	1
	% within "After"	100.0	0	0	100.0
	% within genotype	7.1	0	0	1.8
Don't Know	***Count***	6	9	2	17
	% within "Don't Know"	35.3	52.9	11.8	100.0
	% within genotype	42.9	24.3	33.3	29.8
Total	***Count***	14	37	6	57
	% within "Total"	24.6	64.9	10.5	100.0
	% within genotype	100.0	100.0	100.0	100.0

If we conceder only the patients with a traveling history, we find that 45 out of 57 patients (78.9%) have traveled to Egypt. The rest 12 patients (21.1%) visited other places like, Jordan and Saudi Arabia and in few cases to both Jordan and Egypt (Table 10). Referring to table 10, it can be obviously seen that the majority of the patients with genotype 4 infection and a traveling history, have traveled to Egypt (94.6%) compared to a minor proportion that have traveled to countries other than Egypt (5.4%). On the other hand Genotype 1 and its subtypes as well as the mixed genotypes show no preference regarding the traveling place (Fig. 8).

Table 10: Relationship between genotypes and traveling destination.

		Genotype			Total
		1, 1a, 1b	**4, 4a, 4c/d**	**4+1, 4c/d+1**	
Egypt	***Count***	7	35	3	45
	% within Destination	15.6	77.8	6.7	100.0
	% within genotype	50.0	94.6	50.0	78.9
Other*	***Count***	7	2	3	12
	% within Destination	58.3	16.7	25.0	100.0
	% within genotype	50.0	5.4	50.0	21.1
Total	***Count***	14	37	6	57
	% within Destination	24.6	64.9	10.5	100.0
	% within genotype	100.0	100.0	100.0	100.0

Other: Like Jordan, Saudi Arabia and Emirate*

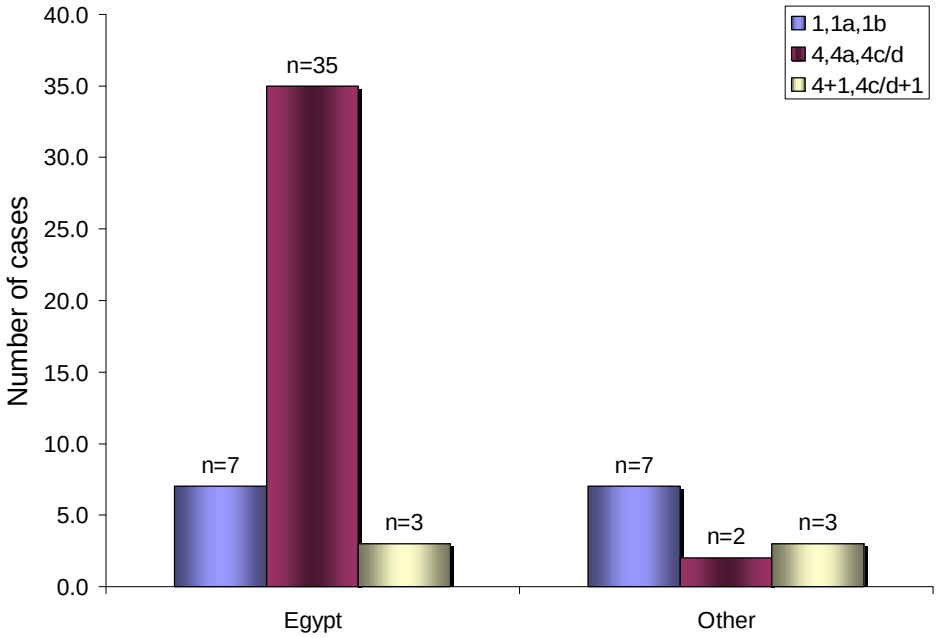

Fig.8: Relationship between HCV genotypes and previous traveling destination of the patients. *The patients with traveling history were classified into 2 main categories based on the traveling destination (X–axis). The distribution of the HCV genotype among each category is shown in the bar-chart. The number of cases is indicated on the top of each bar.*

Relationship between genotype and history of blood transfusion

Table 11 shows that blood transfusion gives a higher chance for mixed infection to occur than either type 1 or type 4 alone. Five out of 7 patients with mixed genotypes (71.4%) have a history of blood transfusion.

From table 11 also it can be seen that the total number of patients that ever had a blood transfusion was 40 patients (43.5%). All of those 40 patients got their blood transfusion before being infected with HCV (Table 11). Moreover, genotype 4 and its subtypes are the prevailing genotypes among those who had the transfusion (60%) as well as among those who did not have the transfusion (67.3%) table (11).

Table 11: Distribution of the different HCV genotypes and subtypes among patients with or without a history of blood transfusion:

		Genotype			Total
		1, 1a, 1b	4, 4a, 4c/d	4+1, 4c/d+1	
Yes	*Count*	11	24	5	40
	% within "Yes"	27.5	60.0	12.5	100.0
	% within genotype	42.3	40.7	71.4	43.5
No	*Count*	15	35	2	52
	% within "No"	28.8	67.3	3.8	100.0
	% within genotype	57.7	59.3	28.6	56.5
Total	*Count*	26	59	7	92
	% within Total	28.3	64.1	7.6	100.0
	% within genotype	100.0	100.0	100.0	100.0

A significant relation could be established between the genotypes and the place at which the patients had their blood transfusion (P value = 0.026). This relation is obvious in the case of Egypt transfusions, as 13 out of 14 patients (92.9%) who had a transfusion in Egypt are infected by the genotype 4 and its subtypes, the dominant genotypes in Egypt. No preference however could be seen between genotypes 1 (40%) or genotypes 4 (44%) for patients who had a transfusion in Gaza Strip (n=25). Only one patient was transfused in Jordan and is infected by genotype 1 (Fig. 9 and Table 13).

Table 12: Distribution of the different HCV genotypes and subtypes among patients with a history of blood transfusion before or after infection.

		Genotype			Total
		1, 1a, 1b	4, 4a, 4c/d	4+1, 4c/d+1	
Before	*Count*	11	24	5	40
	% within "Before"	27.5	60	12.5	100
	% within genotype	100	100	100	100

Table 13: Relationship between genotypes and place of blood transfusion:

		Genotype			Total
		1, 1a, 1b	4, 4a, 4c/d	4+1, 4c/d+1	
Gaza	*Count*	10	11	4	25
	% within "Gaza"	40	44	16	100
	% within genotype	90.9	45.8	80.0	62.5
Egypt	*Count*	0	13	1	14
	% within "Egypt"	0	92.9	7.1	100
	% within genotype	0	54.2	20	35
Jordan	*Count*	1	0	0	1
	% within "Jordan"	100	0	0	100
	% within genotype	9.1	0	0	2.5
Total	*Count*	11	24	5	40
	% within Total	27.5	60	12.5	100
	% within genotype	100	100	100	100

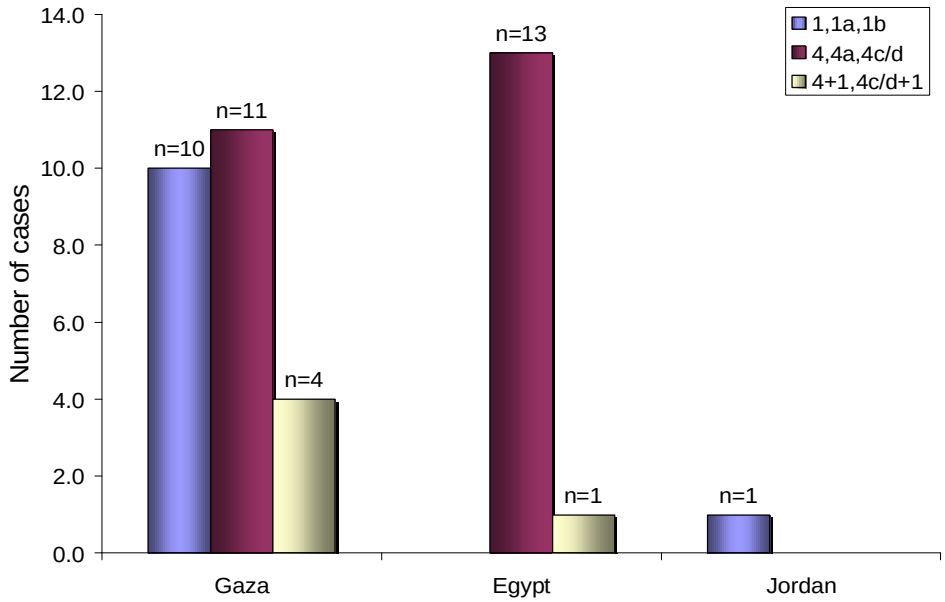

Fig. 9: Relationship between genotypes and places of blood transfusion. *Only patients with a history of blood transfusion were considered and divided into 3 different categories based on the place in which they had their transfusion (x-axis). Distribution of the different HCV genotypes among each category is shown in the bar-chart representation. The number of patients is indicated on the top of each bar.*

Relationship between HCV genotypes and the patient history of surgical operations.

Table 14 describes the relation between histories of surgical operations to the different HCV genotypes. The table shows that in total 48.9% of the patients underwent a medical operation during their life (n=45) compared to 51.1% who haven't undergone any medical operations (n=47). The same situation is seen in each of the genotypes with almost equal number of patients who had or hadn't any operations (table 14). However if we consider only the 45 patients with a history of medical operations we find that almost all of those patients underwent their medical surgeries before being infected with HCV, 86.7% compared to 6.7% after infection and 6.7% that don't know the date (Table 15). This is also true for

each of the different genotypes (78.5% compared to 14.3% and 7.2% for genotype 1 and its subtypes; 88.9% compared to 3.7% and 7.4% for genotype 4 and its subtypes and 100% for the mixed genotypes). Moreover a significant relation could be established between the genotype and the place of medical surgery (P-value = 0.001). For example 17 out of the 18 patients who had their surgeries in Egypt have genotype 4 and its subtypes (94.4%), while slightly more patients with genotypes 1 and its subtypes (50%) than genotype 4 and its subtypes (41.7%) had their surgeries in Gaza. On the other hand, 66.7% of those who had their operations in Jordan have mixed infections (Fig. 10 and Table 16).

Table 14: Distribution of the different HCV genotypes and subtypes among patients with or without a history of surgical operations.

		Genotype			Total
		1, 1a, 1b	**4, 4a, 4c/d**	**4+1, 4c/d+1**	
Yes	***Count***	14	27	4	45
	% within "Yes"	31.1	60	8.9	100
	% within genotype	53.8	45.8	57.1	48.9
No	***Count***	12	32	3	47
	% within "No"	25.5	68.1	6.4	100
	% within genotype	46.2	54.2	42.9	51.1
Total	***Count***	26	59	7	92
	% within Total	28.3	64.1	7.6	100.0
	% within genotype	100	100	100	100

***Table 15: Distribution of the different HCV genotypes and subtypes among patients who underwent a surgical operation before or after infection*.**

		Genotype			Total
		1, 1a, 1b	4, 4a, 4c/d	4+1, 4c/d+1	
Before	*Count*	11	24	4	39
	% within "Before"	28.2	61.5	10.3	100
	% within genotype	78.6	88.9	100	86.7
After	*Count*	2	1	0	3
	% within "After"	66.7	33.3	0	100
	% within genotype	14.3	3.7	0	6.7
Don't Know	*Count*	1	2	0	3
	% within "Don't Know"	33.3	66.7	0	100
	% within genotype	7.1	7.4	0	6.7
Total	*Count*	14	27	4	45
	% within Total	31.1	60.0	8.9	100
	% within genotype	100	100	100	100

Table 16: Distribution of the different HCV genotypes and subtypes within the places of surgical operation

		Genotype			Total
		1, 1a, 1b	4, 4a, 4c/d	4+1, 4c/d+1	
Gaza	*Count*	12	10	2	24
	% within "Place"	50	41.7	8.3	100
	% within genotype	85.7	37	50	53.3
Egypt	*Count*	1	17	0	18
	% within "Place"	5.6	94.4	0	100
	% within genotype	7.1	63	0	40
Other	*Count*	1	0	2	3
	% within "Place"	33.3	0	66.7	100
	% within genotype	7.1	0	50	6.7
Total	*Count*	14	27	4	45
	% within "Place"	31.1	60	8.9	100
	% within genotype	100	100	100	100

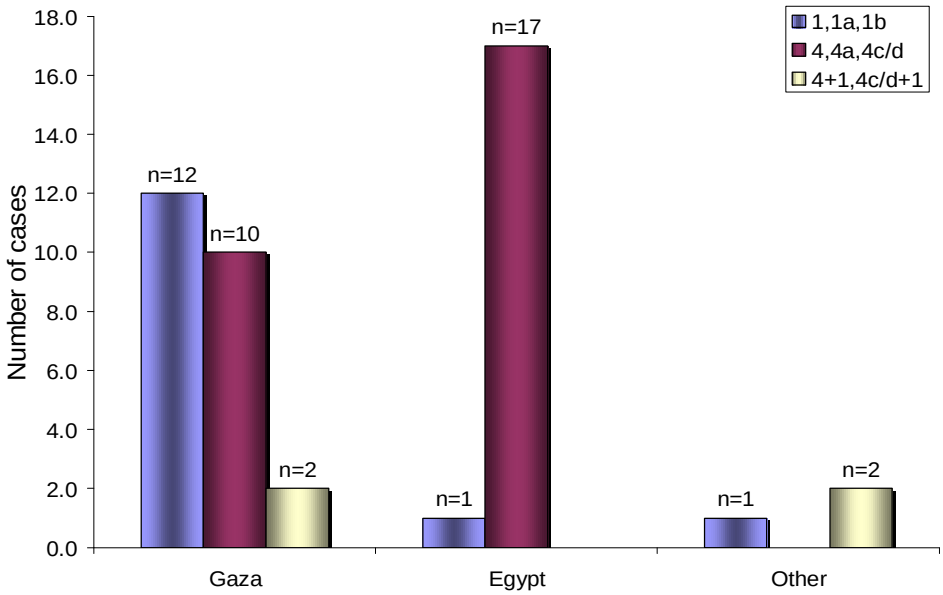

Fig. 10: Relationship between the HCV genotypes and the place of surgery. *Only patients who had a surgical operation were considered, and classified according to the place of surgery (x-axis). The distribution of HCV genotypes is shown within each group of patients with a common place of surgery. The number of patients is indicated on the top of each bar.*

Relationship between HCV genotypes and ALT level in the patient blood

The relation between HCV genotype and ALT level is shown in (Table 17). A significant relation could be established between the number of patients with ALT values above or below 40 IU/ml and the genotype of the patient (P-value = 0.003). For example, 73.1% of the patients infected with genotype 1 and its subtypes (19 out 26) have ALT values above 40 IU/ml (the normal upper limit). On the other hand 66% of the genotype 4 patients (39 out 59), and 71.4% of the mixed genotypes patients (5 out of 7) have ALT values lower than 40 IU/ml. Moreover, if we compare the mean of the ALT values for patients from the different genotypes, we find that patients infected with genotype 1 and its subtypes have significantly higher ALT values than those infected with genotype 4 and its subtypes and the mixed genotypes (P-value ≤ 0.04) (Fig. 11).

Table 17: Distribution of the different HCV genotypes and subtypes among patients having ALT values below or above 40 IU/ml

		Genotype			Total
		1, 1a, 1b	4, 4a, 4c/d	4+1, 4c/d+1	
0-40	*Count*	7	39	5	51
	% within genotype	26.9	66.1	71.4	55.4
Over 40	*Count*	19	20	2	41
	% within genotype	73.1	33.9	28.6	44.6
Total	*Count*	26	59	7	92
	% within genotype	100	100	100	100

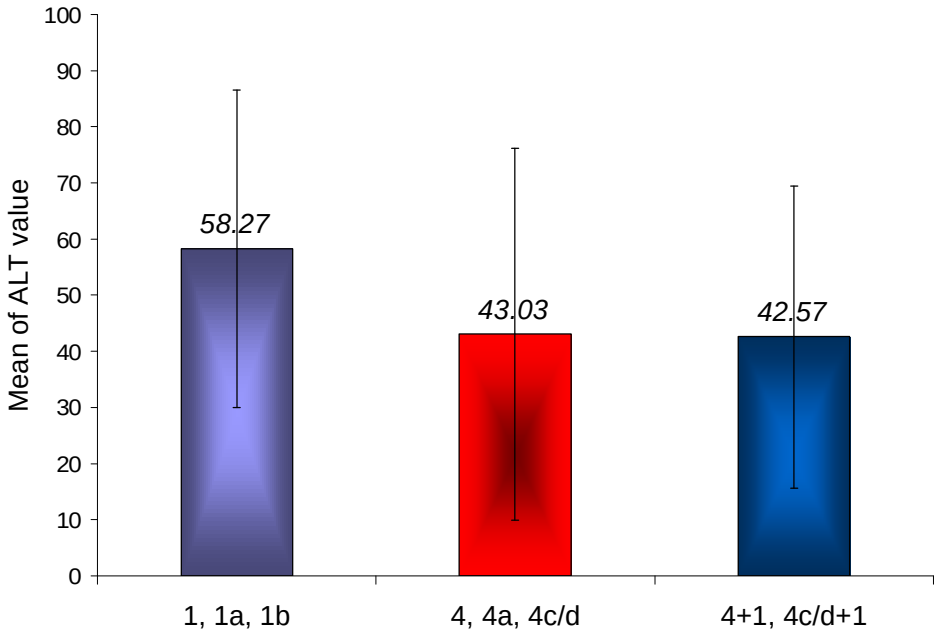

Fig. 11: The relationship between the different HCV genotypes and the mean ALT value*. The mean was calculated for patients from each genotype group and plotted in a bar chart. The means were compared and t-test and standard deviation calculated for each pair at a time.*

Relationship between HCV genotypes and hemodialysis

Fifteen out of the 92 patients (16.3%) undergo dialysis on a regular basis (Table 18). The distribution of the different genotypes among hemodialysis patients is not different from that in the rest of patients. Four hemodialysis patients (26.7%) are infected with type 1 and its subtypes, 9 patients (60%) with type 4 and its subtypes and 2 patients (13.3%) have combined infections. On the other hand, 22 of the rest of patients (28.6%) are infected with type 1 and its subtypes, 50 patients (64.9%) are infected with type 4 and its subtypes and 5 patients (6.5%) have combined infection.

Table 18: Distribution of the different HCV genotypes among hemodialysis and non-hemodialysis patients

		Genotype			Total
		1, 1a, 1b	4, 4a, 4c/d	4+1, 4c/d+1	
Yes	*Count*	4	9	2	15
	% within "Yes"	26.7	60	13.3	100
	% within genotype	15.4	15.3	28.6	16.3
No	*Count*	22	50	5	77
	% within "No"	28.6	64.9	6.5	100
	% within genotype	84.6	84.7	71.4	83.7
Total	*Count*	26	59	7	92
	% within Total	28.3	64.1	7.6	100
	% within genotype	100	100	100	100

Relationship between HCV genotypes and the patients level of education.

The patients were divided into two groups based on the number of years they received their education. The first group (primary) with less than 10 years of education (n=62) accounted for 67.4% of the patients, while the second group (secondary) with more than 10 years of education (n=30) accounted for (32.6%) table (19). No significant relation could be detected between any of the groups and HCV genotypes (P-Value=0.8. The different HCV genotypes are almost similarly distributed within the two groups.

Table 19: Distribution of the different HCV genotypes among patients with primary and secondary levels of education.

		Genotype			Total
		1, 1a, 1b	4, 4a, 4c/d	4+1, 4c/d+1	
primary	*Count*	17	41	4	62
	% within primary	27.4	66.1	6.5	100
	% within genotype	65.4	69.5	57.1	67.3
secondary	*Count*	9	18	3	30
	% within secondary	30	60	10	100
	% within genotype	34.6	30.5	42.9	32.6
Total	*Count*	26	59	7	92
	% within total	28.3	64.1	7.6	100
	% within genotype	100	100	100	100

Relationship between HCV genotypes and infection date

As shown in table (20) there is no significant difference between the infection with any HCV genotype and the date of infection. The mean period of infection for genotype 1, 4 and the mixed genotype is 2.781, 2.3807 and 3.142 respectively. However the difference between these means is not statistically significant (P value=0.8).

Table 20: Distribution of the different HCV genotypes among patients with different durations of infection.

		Genotype			Total
		1, 1a, 1b	4, 4a, 4c/d	4+1, 4c/d+1	
0-1year	*Count*	11	24	2	37
	% within duration	29.7	64.9	5.4	100
	% within genotype	42.3	40.7	28.6	40.2
over 1 year	*Count*	15	35	5	55
	% within duration	27.3	63.6	9.1	100
	% within genotype	57.7	59.3	71.4	59.8
Total	*Count*	26	59	7	92
	% within duration	28.3	64.1	7.6	100
	% within genotype	100	100	100	100

Sequence analysis

The clustering pattern of the different sequences used in the multiple alignment and tree construction was generally informative (Fig. 12). Two major leaves were clustered in the tree. These comprise the type 1 sequences in one leaf and the type 4 sequence in another. The mixed genotypes also clustered in a separate leaf but with some of the type 1 and type 4 sequences.

All of the sequences from patients who had a surgery before being infected were aligned and a tree was constructed. Once again the type 1 sequences clustered separately from type 4 sequences (Fig. 13). Moreover, sequences from patients with a surgery in a given area (like Egypt) clustered together within the same group (they are more closed related). This might indicate a common origin for the infection. The same was also seen with sequence for blood transfusion patients (Fig 14A and 14B).

By referring to (Fig. 13) it can be seen that only one patient who had a surgery in Egypt was infected by genotype 1a, which is the rare genotype in Egypt. The same patient had blood transfusion in Gaza (Fig. 15) which may explain getting a genotype 1a HCV and not 4. Moreover, in all of the trees indicated it can be clearly seen that patients who had a blood transfusion and underwent a surgery in a given country got the genotype that is most common in that country. This establishes a strong link between undergoing a blood transfusion and a surgery in Egypt for example and getting genotype 4 HCV infections. This relation is not accidental, but it seems that patients got the HCV infection as consequence to the surgery and / or blood transfusion.

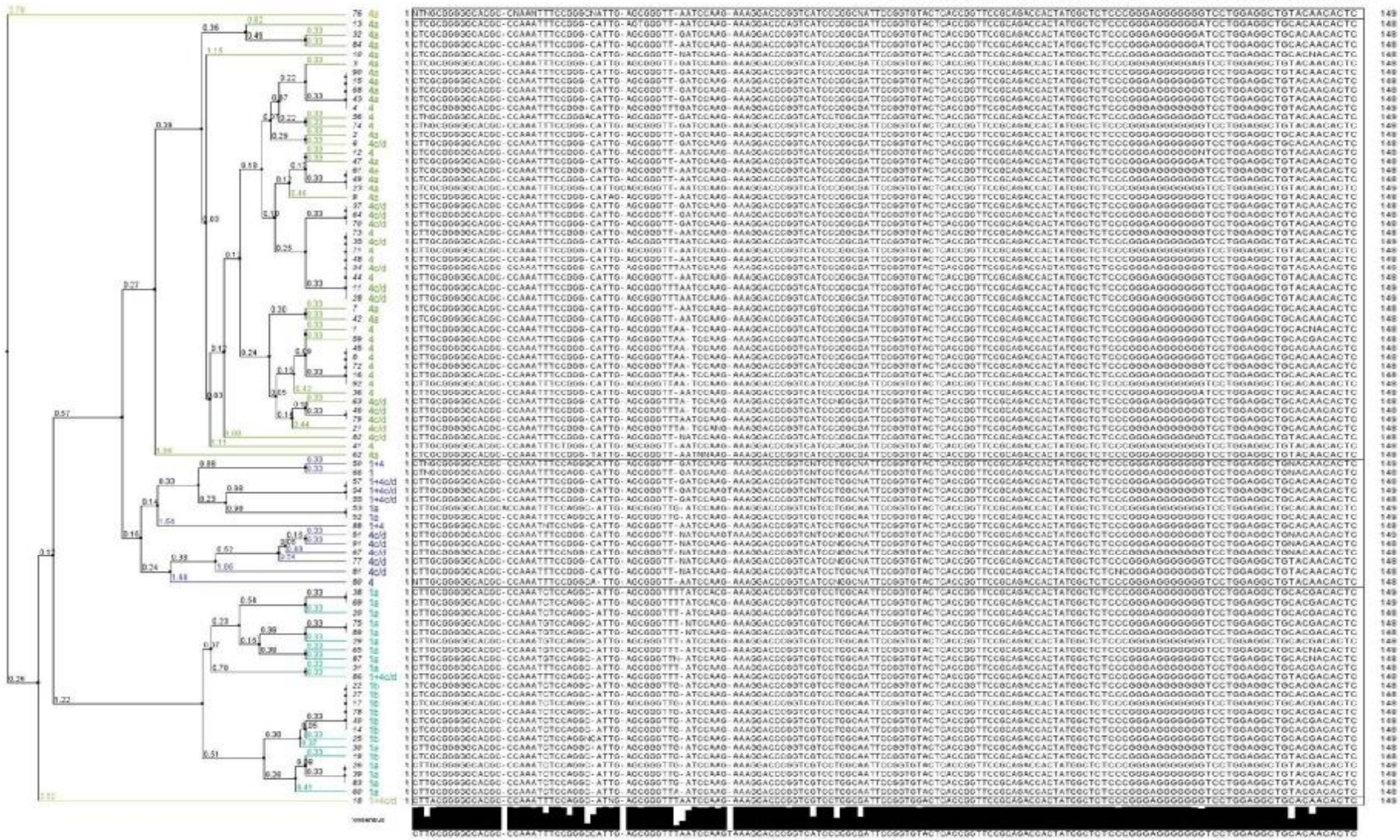

Fig. 12: The clustering pattern and multiple sequence alignment of the different sequences used in the study*. The sequences from patients who were aligned and used for construction of a dendrogram as described in the materials and methods. The genotype for each patient and the distances are indicated.*

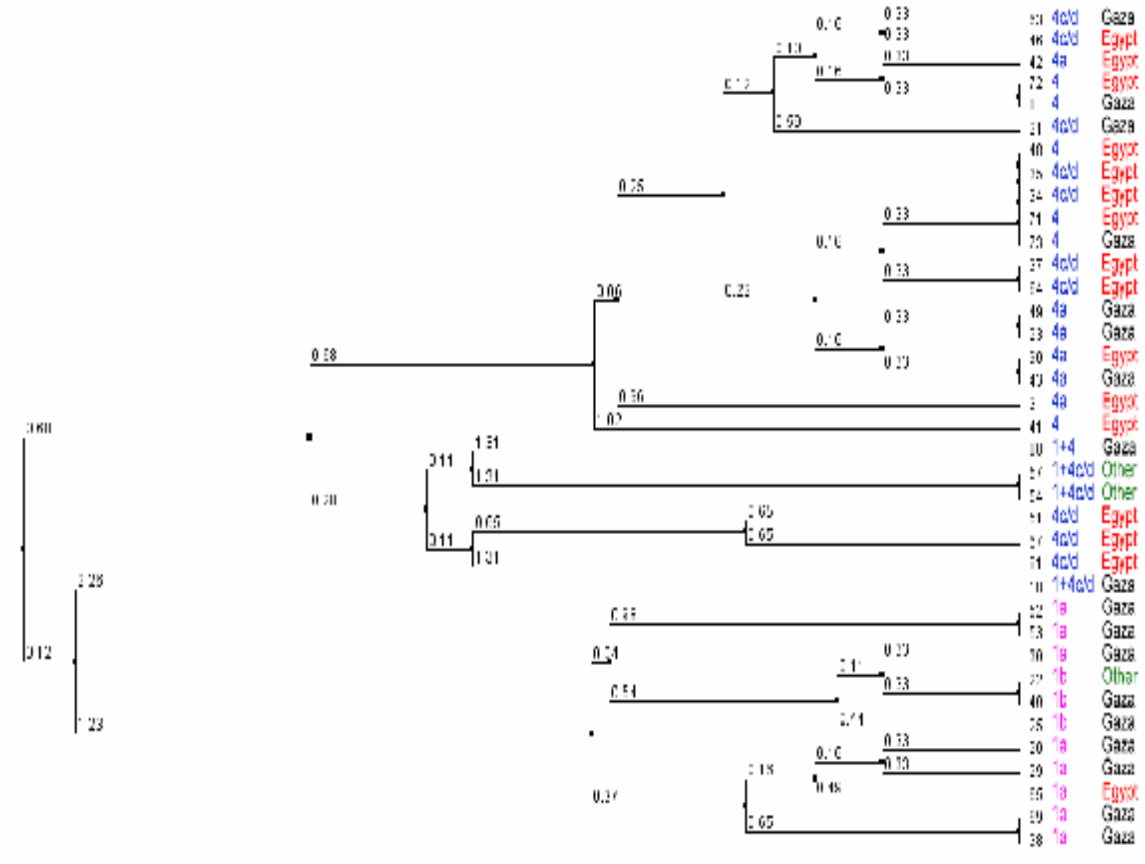

Fig. 13: A dendrogram of sequences from patients with surgery. *Sequences from patients who underwent a surgery before infection were aligned and used for construction of a dendrogram as described previously. The genotype for each patient and the place of surgery are indicated to the right and the distances are indicated.*

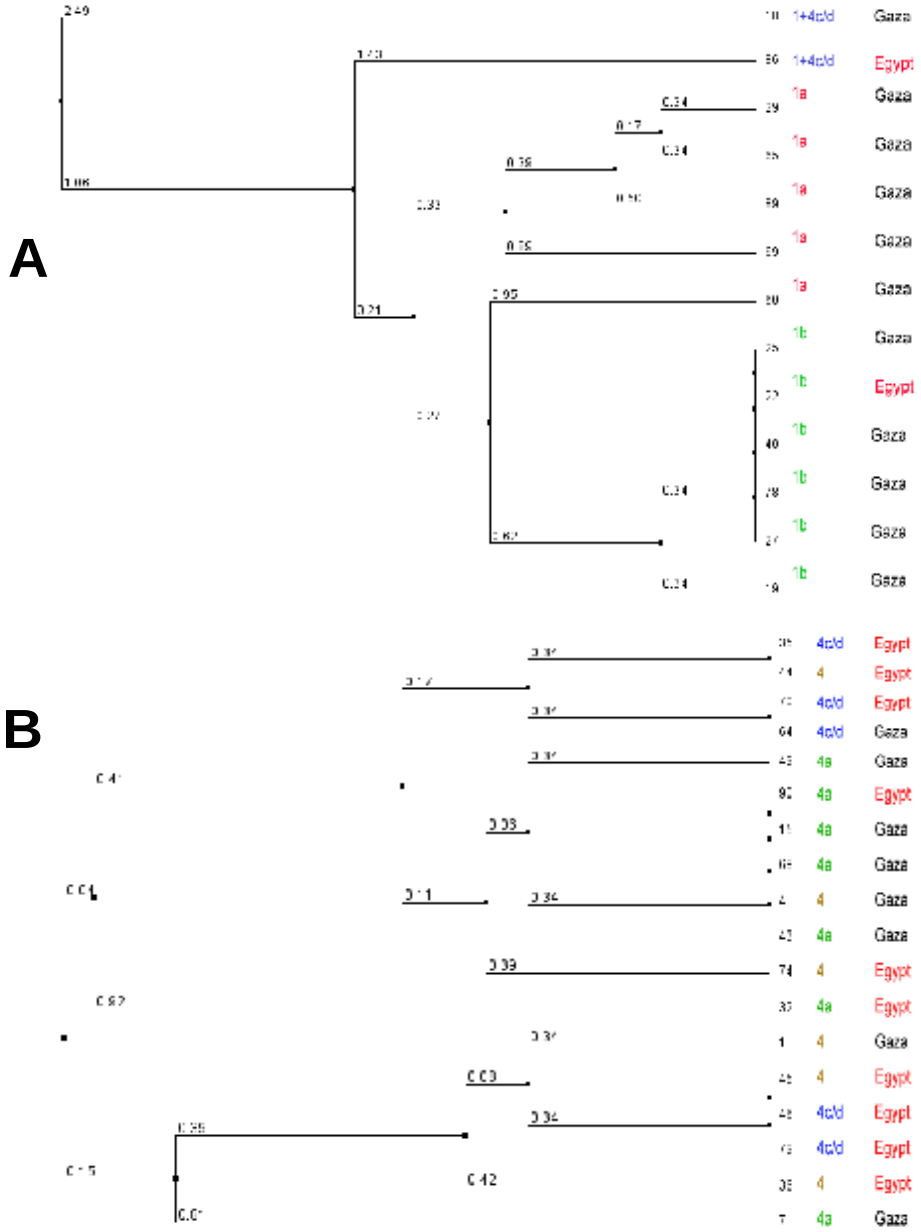

Fig. 14: A dendrogram of sequences from patients with blood transfusion. *Sequences from patients who underwent a blood transfusion before infection were aligned and used for construction of a dendrogram as described previously. The genotype for each patient and the place of transfusion are indicated to the right and the distances are indicated. (**A**) Transfusion before infection with type 4. (**B**) Transfusion before infection with type 1.*

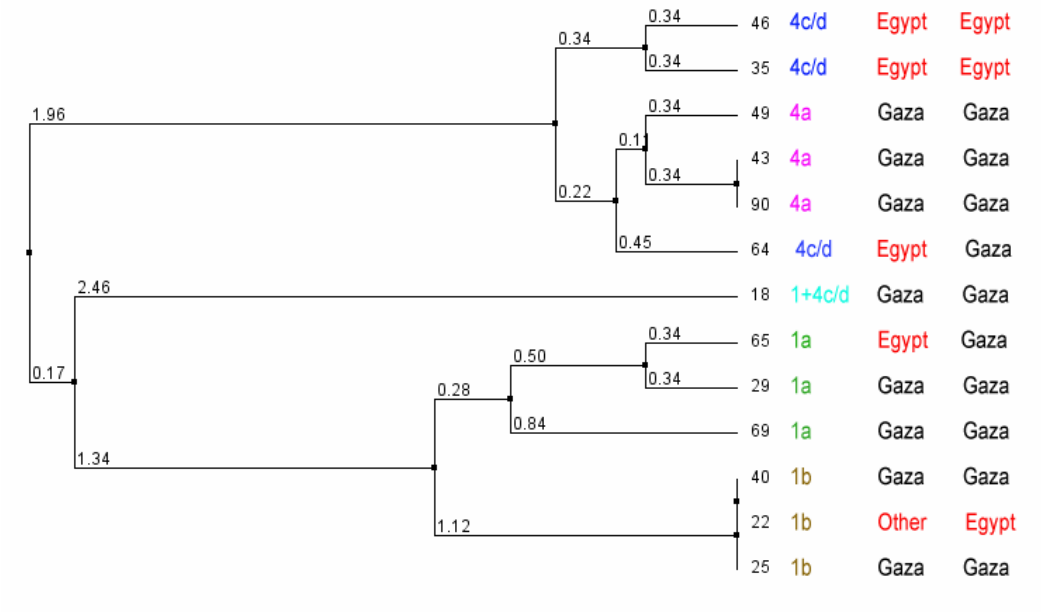

Fig. 15: A dendrogram of sequences from patients with both blood transfusion and surgery. *Sequences from patients who underwent both a blood transfusion and surgery before infection were aligned and used for construction of a dendrogram as described previously. The genotype for each patient and the place of transfusion (to the right) and surgery (in the middle) are indicated and the distances are indicated.*

Summary of the main risk factors evaluated in the study:

Traveling, surgery and blood transfusion are the main roots of HCV transmission in Gaza strip but are not the only ones (Fig 16). Other roots of transmission must be present as 14 patients (15.2%) were not included in any of these categories. A combination of two or more of these factors will increase the risk. For example, local surgeries contribute only to 7.6%, while if conducted abroad the risk doubles (18.5%). The same is also true for blood transfusion who contributes to 8.7% if performed locally compared to 13% when performed abroad.

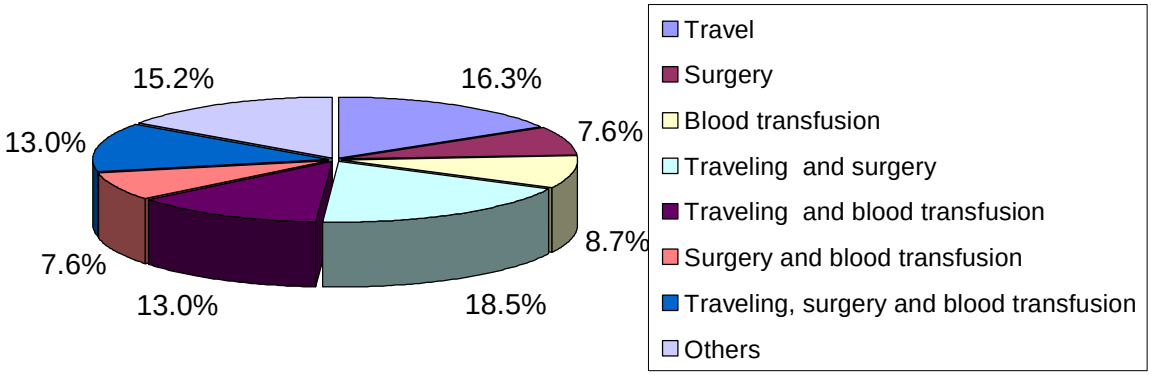

Fig. 16: Summary of risk factors associated with HCV transmission in Gaza Strip

DISCUSSION

Hepatitis C is caused by a small RNA virus that belongs to the family flaviviridae and is the sole member of the genus hepacivirus. First identified in 1989, the HCV has a single-stranded 9646-bp RNA genome that encodes a single, large polyprotein. The HCV polyprotein is cleaved post-translationally into multiple structural and non-structural peptides. HCV is efficiently transmitted by direct precutaneous exposure to infectious blood, sexual transmission, intravenous drug use, needle stick accidents, and blood transfusions before hepatitis C screening (2, 31).

Over 170 million people around the world are infected with hepatitis C and are at risk of developing cirrhosis and liver cancer (5, 21). Currently, HCV is one of the main causes of liver-related morbidity and mortality, being responsible for an estimated 8,000 to 10,000 deaths annually in the world (4). According to the Palestinian ministry of health, the prevalence of HCV among blood donors in Palestine was estimated to be 0.3% in 2003 and 0.2% in 2004 (20). However, this estimation was based on a defined group of the Palestinian people which is the blood donors. These usually constitute the healthiest group of people who are mostly males in the midlife age. Moreover, these figures are also based on screening techniques that involve the detection of Anti-HCV antibodies by the ELISA method. As mentioned earlier false-positive results may be given after the infection has resolved as the antibody titer remains high, and in individuals with hypergammaglobulinemia, as a result of autoimmune hepatitis (69). False negative results are also obtained in the first 3-4 months window-period needed for the infected person to mount detectable levels of anti-HCV antibodies and in immunocompromised patients (70). Thus these figures are likely to be underestimated, and HCV prevalence in Palestine might be higher than the reported one. This fact is supported by the results of a cross-sectional study of 399 health personnel conducted in governmental healthcare settings of the southern region of Gaza Strip in 2003 (80). The study showed that: The prevalence of anti-HCV was 1.3% among health workers.

HCV is known to have a marked genetic heterogeneity that results from point mutations, single nucleotide substitutions, deletions or insertions introduced to the viral genome during its replication by the viral encoded RdRp (5, 34). At least six major HCV genotypes and more than 50 HCV subtypes differ by as much as 30% to 50 % in their nucleotide sequences (7, 42).

The determination of HCV genotypes usually has prognostic and therapeutic values. There is increasing evidence that patients infected with different HCV genotypes may have different clinical profiles, severity of liver disease, and response to anti-HCV therapy (27). Moreover, HCV genotyping may shed light on its evolution, source of outbreaks, and risk factors. It may be used to identify the source of infection in cases of patient-to-patient transmission and is also useful in the study of other modes such as vertical (mother to baby), sexual transmission and needle stick injury (28, 29).

Currently, there is almost no available data about the most prevalent HCV genotypes in the Palestinian territories. Moreover, genotyping of the HCV is not requested by Palestinian physicians as a part of the disease assessment protocol or therapy regiments of HCV patients in the Palestinian territories. Therefore this study concentrates on the identification of the most common HCV genotypes and subtypes in Gaza strip in order to shed some light on the potential significance of this issue. In addition, some other aspects of HCV, like its mode and origin of transmission in Gaza strip have been evaluated by analyzing the data obtained for HCV genotype and the patient personal information collected by a questionnaire. The study also analyzes some risk factors for acquiring HCV in Gaza strip such as, travelling, blood transfusion and surgery in endemic areas.

The accumulation of nucleotide substitutions in the HCV genome has resulted in its diversification and the evolution of different HCV genotypes (27). Although a variety of methods have been used for HCV genotyping, sequencing of an informative region of its genome remains the golden standard. This method usually depends on the detection of certain nucleotide differences between the different genotypes in the 5'UTR region. The nucleotide differences that are

characteristic of a certain genotype have to be maintained by all members of that genotype and not easily changed each time the virus replicates.

As previously mentioned, the 5'UTR is highly conserved, and therefore is very suitable for both RT-PCR detection and genotyping of HCV. Regarding the RT-PCR diagnosis we usually need a primer binding site that is universal to all genotypes so that the test can't miss any one. Such locations can only be found within the 5'UTR region of the 9646-bp HCV genome. The highly conserved 5'UTR region is also ideal for determining the HCV genotypes because the characteristic nucleotides of each genotype are rarely changed by random chance in this region due to its conservation.

However, the 5'UTR is not usually good enough in reflecting the relatedness of HCV isolates and lineages that evolve within the same genotype. This is also due to its high conservation. Therefore, scientists usually compare sequences from highly variable regions of the viral genome (like E1 and E2) or they may use the whole genome sequence for this purpose.

Unfortunately sequences from these variable regions of the genome of the HCV viruses studied in this work were not available. Moreover, if we wish to determine such sequences, we would need a dedicated another PCR and sequencing procedure for the hypervariable region, which was not possible. Therefore, we used the available sequences from this study (from 5'UTR) to roughly estimate the relatedness of the different sequences to each other by sequence comparison and dendrograms. Not surprisingly we found a great level of homology between the compared sequences, and the only differences were so few and confined to certain locations of the 5'UTR (fig. 12). Moreover, as will be discussed later, sequences from this region were enough to reflect the general clustering pattern of the sequences studied to a certain degree.

The results of this study indicate that there are only two major genotypes of HCV in Gaza Strip: Genotype 1 and its subtypes 1a and 1b, and genotype 4 and its subtypes 4a and 4c/d (Table 5). Mixed infection with the two genotypes were also seen and included a combination of genotypes 1 and 4 or 1 and 4c/d. HCV

genotype 4 and its subtypes are the predominant genotypes in Gaza strip (64.1% of the cases).

Genotypes 1 and 4 were previously reported to be more pathogenic and considered more difficult to treat. For example the rate of progression to chronicity after acute exposure to HCV was significantly higher in patients exposed to HCV genotypes 1b and 4 infections than in patients exposed to other genotypes (3, 47). Furthermore, higher incidence of HCV genotype 1b was detected in patients with HCV-associated hepatocellular carcinoma and decompensates liver disease, requiring liver transplantation, than among those with chronic active HCV (46, 47and 48). Also HCV genotype 4 patients have a worse prognosis compared to other HCV genotypes after liver transplantation (14). Moreover patients infected with type 1 and 4 had a higher viral load than patients infected with other genotypes (46).

Research has shown that patients with genotype 1 and 4 show lower SVR than patients with other genotypes (46, 48 and 60). Genotype 1 and 4 infections require therapy for 48 weeks, whereas shorter treatment is feasible in genotype 2 and 3 infections (8, 64).

The two major HCV genotypes (1 and 4) were found to be significantly distributed among patients from the south and north of Gaza strip (P-value = 0.01). Genotype 4 and its subtypes are concentrated in the south of Gaza strip (71.2%), while genotype 1 is slightly more represented in the north (57.7%) than in the south (42.3%) of Gaza strip (Table 7).

It is well documented that genotype 4 and its subtypes especially 4a are the predominant genotype in Egypt, the southern border of Gaza strip and the Gaza only gateway to other parts of the world. Many Gaza residents, especially from the south, have historical relationships with the Egyptian people. Moreover, members of many families were divided between Gaza and Egypt, but keeping strong social links with frequent visits from both sides. On the other hand, Israel with the most predominant genotype 1 and its subtypes borders Gaza strip from the north giving the northern Gaza residents more chance to work in Israel.

Moreover, the Egyptians ruled Gaza strip in the period between 1948 and 1967 and Israel occupied Gaza at 1967 and controlled the Palestinian lives until the establishment of the Palestinian National Authority. Based on the upper mentioned information, the genotypes distribution in the south and north of Gaza and each of Egypt and Israel may be correlated. This assumption may raise clues about the rout of HCV introduction to Gaza strip.

As we previously mention, traveling abroad is associated with increased risk of HCV infection in Gaza strip. The risk is expected to be more modifiable when traveling is to endemic areas of the world (81). Our results of the study show that patients who have traveled to a foreign country (62.0%) are almost twice those who haven't (38.0%) (Table 8). The majority of the patients with a traveling history (68.4%) traveled before they were infected (Table 9). Only one patient (1.8%) reported that he traveled after he was infected, and 17 patients (29.8%) don't know if they traveled before or after the infection (table 9).

It is likely that traveling has contributed more to genotype 4 and its subtypes (62.7% "Yes" compared to 37.3% "No") and to the mixed genotypes (85.7% "yes" compared to 14.3% "No"), than to genotype 1 and its subtypes (53.8 %" yes" compared to 46.2% "No").

If we only conceder patients with a traveling history, we find that 78.9% of them have traveled to Egypt, which shows one of the highest HCV prevalence in the world (13, 14). The majority of the patients who traveled to Egypt (77.8%) have genotype 4 and its subtypes, while only 16.7% of the patients who traveled to other countries are infected with this genotype (Table 10). If we put it another way, we can say that the great majority (94.6%) of patients with genotype 4 infections and a traveling history, have traveled to Egypt (Table 10). The relationship between the genotypes distribution and the traveling destination is statistically significant (P-value = 0.005).

Surgery is another risk factor for nosocomial HCV transmission in a health care sitting (41), a factor that was also evaluated in this study. The results show that almost half of the patients participating in the study (48.9%) had a surgical

operation, of which 84.8% had their surgery before the infection with HCV (table 14 and 15). Furthermore, a significant relation between the place of surgery and HCV genotypes was observed (P value = 0.001). For example, 94.4% of the patients who had their surgeries in Egypt have genotype 4 and its subtypes, the major subtype in Egypt (Table 16). This suggests that those patients might have got infected during the surgery. On the other hand, slightly more patients with genotype 1 and its subtypes (50%) than genotype 4 and its subtypes (41.7%) had their surgeries in Gaza, and 66.7% of those who had their operations in other places have mixed infections (Table 16).

The suggested possible transmission of HCV as a result of surgery may be supported by the fact that sequence from patients with a surgery in a given area (like Egypt) are usually more related (they cluster together within the same leaf of dendrogram) (Fig. 13).

During blood transfusion, there is a higher risk to get HCV, especially when laboratory screening techniques are absent or fail to detect HCV infection in a fraction of blood donations. This makes HCV responsible for about 90%-95% of transfusion-associated hepatitis cases (2, 31).

The results show that all of the patients with a history of a blood transfusion (43.5%) got their blood transfusion before being infected with HCV (Table 11). Table 11 shows that blood transfusion gives a higher chance for infection with mixed genotypes than with either type 1 or type 4 alone. Five out of 7 patients with mixed genotypes (71.4%) have a history of blood transfusion.

A significant relation could be established between HCV genotypes and the place of transfusion (P-value = 0.026). This relation is obvious in the case of Egypt transfusions, as 13 out of 14 patients (92.9%) who had a transfusion in Egypt are infected by the genotype 4 and its subtypes, the dominant genotypes in Egypt. No preference however could be seen between genotypes 1 (40%) or 4 (44%) for patients who had a transfusion in Gaza Strip.

Double infection is present in 5 of 40 patients (12.5%) who had received blood. Those patients may be exposed to repeated blood transfusion or infected

by another rout of HCV transmission. Like in surgery, these data may be supported by sequence comparison and dendrograms (Figure 14A, and 14B).

By referring to (Fig. 12) it can be seen that one patient who had surgery in Egypt was infected by genotype 1a, the rare genotype in Egypt. This may be explained by the fact that this patient had a blood transfusion in Gaza from which he might have gotten his infection (Fig. 15). Based on sequence relatedness, it can be postulated that patients who had a blood transfusion and underwent a surgery in a given country got the genotype that is most common in that country (Fig. 15). This establishes a strong link between undergoing a blood transfusion and a surgery in Egypt for example and getting genotype 4 HCV infections. This correlation is not accidental and patients got the HCV infection as consequence to the surgery or transfusion.

ALT is a liver enzyme that is more specific for liver damage than other enzymes (72). A significant relation could be established between the number of patients with ALT values above or below 40 IU/ml and the genotype of the patient (P-value= 0.003). For example, 73.1% of the patients infected with genotype 1 and its subtypes (19 out 26) have ALT values above 40 IU/ml (the normal upper limit). On the other hand 66% of the genotype 4 patients (39 out 59), and 71.4% of the mixed genotypes patients (5 out of 7) have ALT values lower than 40 IU/ml. Moreover, if we compare the mean of the ALT values from genotype 1 patients and genotype 4 patients we find that patients infected with genotype 1 and its subtypes have significantly higher ALT values than those with genotype 4 and its subtypes (P-value= 0.04) (Fig.11).

These results suggest that patients infected with genotype 1 are more susceptible to liver complications than those infected with genotype 4. These results are in consistence with published data that correlate higher incidence of HCV genotype 1b with HCV-associated hepatocellular carcinoma, decompensate liver disease and the need for liver transplantation (46, 47and 48). These data should be considered when evaluating the patient for therapy, and thus imply that genotyping should be part of HCV diagnostic protocol.

There is no statistically significant relationship between the occurrence of a certain genotypes and age, education level and date of infection. Moreover, most of the patients didn't know when they got the infection. This may be explained by the fact that only about 20%-30% of the newly infected persons show symptoms of acute hepatitis, while chronic infections, mostly asymptomatic, develop in 75%-85% of adult infected persons and in 50%-60% of juveniles (4).

As previously mentioned, hemodialysis patients are at high risk of nosocomial HCV transition, and being repeatedly exposed to this risk increases the chance for infection with multiple HCV genotypes. The results of the study don't show statistically significant relationship between HCV genotypes and dialysis. The presence of double genotype infection was reported only in 2 patients indicating a possible nosocomial transmission of HCV during dialysis.

Traveling, surgery and blood transfusion are the main roots of HCV transmission in Gaza strip but are not the only ones (Fig 16). Other roots of transmission must be present as 14 patients (15.2%) were not included in any of these categories. A combination of two or more of these factors will increase the risk.

CONCLOUSION

From this study, we conclude that genotype 1 and 4 and their subtypes are the major common HCV genotypes in Gaza strip, and Genotype 4 and its subtypes especially 4a are the predominant ones.

The results must be considered in tailoring treatment schedules and monitoring of antiviral treatment in accordance with genotypes 1 and 4 suitable regiments. Genotyping newly discovered infections must be performed as a part of the diagnostic protocol as patients with genotype 1 may show increased risk for liver damage than genotype 4.

Travelling, surgery and blood transfusion in an endemic HCV area are the major roots of HCV transmission in Gaza strip although other roots exist that were or were not evaluated in this study.

Egypt is probably the major source of Genotype 4 HCV infection in Gaza Strip via one or more of the upper mentioned roots and thus southern Gaza residents have more chance for getting HCV genotype 4 and its subtype than in the northern residents.

Mixed infections are more likely to be caught by old patients, patients travelling abroad and/or having blood transfusion.

The results showed that genotype 1 infections are more serious than genotype 4 infections with higher ALT levels.

The education level, infection date and hemodialysis don't affect the HCV genotype and its subtype infection.

Finally we can conclude that, the results will contribute to the identification of sources of outbreaks, risk factors and control strategy. The results will also help to tailor treatment schedules and monitoring effect of antiviral treatment.

RECOMMENDATIONS

Based on the results of the study we highly urge the decision makers in the Palestinian ministry of health and other health service providers to adopt the following recommendations:

1. Introducing HCV genotyping service as a part of the diagnostic and prognostic protocol for treatment of HCV infected patients in Palestine.

2. Conducting qualitative PCR or other sensitive tests for screening of HCV before traveling, surgery or blood transfusion in a foreign country to avoid any controversy regarding the transmission of HCV to patients seeking cure at these countries.

3. Adopting more sensitive HCV screening techniques, such as qualitative PCR, for routine screening of blood units in the different blood banks in Palestine to avoid transfusion-associated transmission of the virus.

REFERENCES

1) Center for Disease Control and Prevention (CDC), 2003-Prevention and Control of Infections with Hepatitis_Viruses in Correctional Settings, MMWR, Vol. 52, No. RR-1.

2) Robbins S., Cotran R., Kumar V., 2002-Robbins Pathologic Basis of Disease. W.B. Saunders Company; 5th ed, p 847.

3) Nizar N., 2000- Clinical significance of hepatitis C virus genotype. Clin Microbiol Rev, Vol. 13, No. 2: 223–235.

4) Sulkowski M., Wasserman R., Brooks L., Ball L. and Gish R., 2004-Changes in hemoglobin during interferon alpha-2b plus ribavirin combination therapy for chronic hepatitis C virus infection. J viral Hepat, Vol.11: 243.

5) Kedziora P., Figlerowicz M., Formanowicz P., Alejska M., Jackowiak P., et al., 2005-Computational methods In diagnosis of chronic hepatitis C. J Bull.Pol.Ac.Tech, Vol. 53, No. 3 : 273-274.

6) Bartenschlager R., and Lohman V., 2000-Replication of hepatitis C virus. J Gen Virol, Vol. 81: 1631.

7) Decker R., Trooman H., 1997-Hepatitis C: Essays and Expert Opinion on its Natural History, Epdemiology, Diagnosis and Therapy. Abbott Diagnostics Division, Germany,

8) NIH Consensus Statement on Management of Hepatitis C: 2002-NIH Consensus and State-of-the-Science Statements. Vol. 19, No. 3.

9) Drusano G., and Preston L., 2004-A 48-Week Duration of therapy with pegylated interferon 2b plus ribavirin may be too short to maximize long-term response among patients infected with genotype-1 hepatitis C virus. J Infect Dis, Vol. 189: 964.

10) Germer J., and Zein N., 2001- Advances in the molecular diagnosis of hepatitis C and their clinical implications. Mayo. Clin. Proc, Vol. 76, No. 9: 911.

11) Yeh C-T., 2004-Establishment of authentic HCV replication system for drug screening. J Gast and Hep, Vol.19: 315 – 316.

12) Mokhtar N., Mansour M., El-Borai M., Mansour O., and Zaglool W., 2000-In situ localization of PCR amplified cDNA for the diagnosis of HCV Infection in Egyptian liver biopsies. J Egyptian Nat.Cancer Inst, Vol.12, No. 3: 229.

13) Pybus OG., Drummond AJ., Nakano T., Rbertson., and Rambaut A., 2003-The epidemiology and iatrogenic transmission of hepatitis C virus in Egypt: a Bayesian coalescent approach. Mol. Biol. Evol, Vol. 20, No. 3: 381.

14) Abdo A., and Lee S., 2004-Management of Hepatitis C Virus Genotype 4. J Gast and Hep, Vol.19: 1233-1234.

15) Othman B., and Monem F., 2003-prevelance of hepatitis C virus antibodies among health care workers in Damascus, Syria. Saudi Med. J., Vol. 24, No.7:130.

16) Bakir F**.,** 2003**-**Age-specific prevalence of antibody to hepatitis C virus among the Saudi population. Saudi Med. J., Vol. 24, No.7:136

17) Said R., Hamzeh Y., Mehyar N., and Rabah M., 2003- Hepatitis C virus infection in hemodialysis patients in Jordan**.** Saudi Med. J., Vol. 24, No.7: 138.

18)Glikberg F., Brawer-Ostrovsky J., and Ackerman Z., 1997- Very high prevalence of hepatitis B and C in Bukharian Jewish immigrants to Israel. J Clin. Gastroenterol, Vol. 24, No.1: 30.

19) Health Status in Palestine: Ministry of Health Annual Report 2003, State of Palestine Ministry of Health (Palestinian health information center), Gaza.

20) Health Status in Palestine: Ministry of Health Annual Report 2004, State of Palestine Ministry of Health (Palestinian health information center) Gaza.

21) Drazan KE., 2000-Molecular biology of hepatitis C infection. J Liver Transpl, Vol. 6, No. 4: 396.

22) Highleyman L., 2004-Genotype 3 HCV may resolve spontaneously. A Bi-monthly publication of the hepatitis support project, Vol.1, No.12 : 1.

23) Cochrane A., Searle B., Hardie A., Robertson R., Delahooke T., Cameron S., Teddar R., and Dusheiko G., 2002-A Genetic analysis of hepatitis C virus transmission between injection drug users. J Infect Dis, Vol.186: 1212-1214.

24) Hu Y-W., Balaskas E., Furione M., Yen P., Kessler G., Scalia V., Chui L., and Sher G., 2000-Comparison and Application of a Novel Genotyping Method, Semiautomated Primer-Specific and Mispair Extension Analysis, and Four Other Genotyping Assays for Detection of Hepatitis C Virus Mixed-Genotype Infections. J Clin. Microbiol., Vol. 38, No. 8 :2807

25) Matsubara T., Ryo S., Kenji S., Yoji N., and Hitoshi T., 1996-Genotyping of hepatitis C virus: coinfection by multiple genotypes detected in children with chronic posttransfusion he patitis C. J Pediatr. Gastroenterol. Nutr, Vol. 22, No. 1: 79.

26) Wejsta R., Alaeus A., Fischer B., Reichared O., and Uhnoo I., 2003-Chronic Hepatitis C: Updated Swedish Consensus. J Infect Dis, Vol. 35: 445.

27) Ohno O., Mizokaml M., Wu R.,Saleh M., Ohba K., and Orito E., 1997-New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J Clin. Microbiol, Vol. 35, No.1: 201.

28) Hepatitis C: Prevention and treatment: http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index5.html (last visited on 13/10/2005).

29) Shobokshi O., Serebour F., and Skakni L., 2003-Hepatitis C genotypes / Subtypes among chronic hepatitis patients in Saudi Arabia. Saudi Med J .Vol. 24: 587-588.

30) Yasui K., Wakita T., Tsukiyama-kohara K., Funahashi S., Ichikawa M.,et al., 1998-The native form and maturation process of hepatitis C virus core Protein. J Virol. Vol.72, No. 7: 6048-6055.

31) Feitelson MA., Hepatitis C Virus. Cambridge University Press, UK, 2002, p 3, 14, 20, 145, 148 and 149.

32) Worman H.J.,1997-Molecular biological methods in diagnosis and treatment of liver diseases. Clin. Chem, Vol. 43: 1476.

33) Yi M., and Lemon S., 2003-3′ Nontranslated RNA signals required for replication of hepatitis C virus RNA. J Virol. Vol. 77, No. 6: 3557-3559.

34) Penin F., Dubuisson J., Rey F., Moradpour D., and Pawlotsky J., 2004-Structural biology of hepatitis C virus. J Hepat, Vol.39, No.1: 5- 7.

35) Center for Disease Control and Prevention (CDC), 1998-Recommendations for Prevention and Control of Hepatitis C Virus (HCV) Infection and HCV-Related Chronic Disease, MMWR, Vol. 47, No. RR-19.

36) Taylor D., Shi S., Romano P., Barber G., and Lai M., 1999-Inhibition of the Interferon-Inducible Protien Kinase PKR by HCV E2 Protein. J Science, Vol. 285, No. 5424: 107.

37) Zein N., 2003-The epidemiology and natural history of hepatitis C Virus infection. J of Medicin, Vol. 70: 52 -54.

38) Mozer-Lisewska I., Siuzewski W., Yousef K., Figlerowicsz M., and Piaskowsk K., 2003-Virus genotype 1b and long-term response to

interferon alpha monotherapy in children with chronic hepatitis C. Eur J Pediatr, Vol.162 : 755-756.

39) Moreira R., Pinho J., Fares J ., Oba I., Cardoso M., et al., 2003-Prospective study of hepatitis C virus infection in hemodialysis patients by monthly analysis of HCV RNA and antibodies. J Microbiol. Vol. 49: 503-505.

40) Kokubo S., Horii T., Yonekawa O., Ozawa N., and Mukaide M., 2002-*A* phylogenetic-tree analysis elucidating nosocomial transmission of Hepatitis C Virus in a haemodialysis unit. J Viral Hepat, Vol. 9: 450-451.

41) Tallis G., Ryan G., Lambert S., Bowden D., McCaw R.,et al., 2003-Evidence of patient-to-patient transmission of hepatitis C virus through contaminated intravenous anaesthetic ampoules. J Viral Hepat, Vol. 10 : 234

42) Lawrence M., Tierney J., Stephen J., and Maxine A., 2002-Current Medical Diagnosis and Treatment. Appleton & Lange, P 681.

43) Bean P., and Olive M., 2001-Molecular principles underlying hepatitis C virus diagnosis. Am. Clin. Lab, Vol. 20, No. 5: 19.

44) Busek S., and Oliveira G., 2003-Molecular epidemiology of the hepatitis C virus in Brazil. Genet. Mol. Res, Vol. 2, No. 1: 117.

45) Pawltosky JM., Prescott L., Simmonds P., Pellet C., Laurent-puig P., et al., 1997-Serological determination of hepatitis C virus genotype: comparison with a standardized genotyping assay. J Clin. Microbiol, Vol. 35, No. 7: 1734.

46) Sreevatsan S., Bookout J., Ringpis F., Pottathil M., Marshall D.,et al., 1998-Algorithmic approach to high-throughput molecular screening for alpha interferon-resistant genotypes in hepatitis C patients. J clin. Microbiol, Vol. 36, No.7: 1895.

47) Watson J., Al-Mardini H., Awadh S., Ukabam S., and Record C., 1999-Hepatitis C virus genotypes in a chronic of Middle Eastern patients. J Ann. of Saudi Med, Vol.19, No. 5: 410.

48) Zein NN., Rakela J., Krawitt E., Reddy R., Tominaga T., et al., 1997-Hepatitis C virus genotypes in the United States: epidemiology, pathogenicity, and response to interferon therapy. Collaborative Study Group. Ann. Intern. Med, Vol. 125, No. 8: 634.

49) Nunez M., and Soriano V., 2005-Hepatitis C virus (HCV) genotypes and disease progression in HIV/HCV-coinfected patients. J Infect Dis, Vol. 191, No. 1:1.

50) Hezode C., Roudot-Thoraval F., Zafrani E., Dhumeaux D., and Pawlotsky J., 2004-Different mechanism of steatosis in hepatitis C virus genotypes 1 and 3 infections. J Viral Hepat, Vol. 11: 455-456.

51) Qiao M., Murata K., Davis A., Jeong S., and Liang T., 2003-Hepatitis C virus-like particles combined with novel adjuvant systems enhance virus –specific immune responses. J Hepat. Vol. 37, No. 1: 52.

52) Strader D., Wright T., Thomas D., and Seeff L., 2004-Diagnosis, Management, and Treatment of Hepatitis C. J Hepat, Vol. 39, No. 4: 1147, 1152 and 1155.

53) Wejstal R., Alaeus A., Fischler B., Reichard O., and Uhnoo I., 2003-Chronic hepatitis C: Updated Swedish consensus. J Infect Dis. Vol.35: 446.

54) Pawlotsky J and Mchutchison J., 2003- Hepatitis C. Development of New Drugs and Clinical Trials: Promises and Pitfalls, J Hepat, Vol. 39, No. 2: 555,559 and 556.

55) Hofer H., Watkins-Riedel T., Janata O., Penner E., Holzmann H.,et al., 2003-Spontaneous viral clearance in patients with acute hepatitis C can be predicted by repeated measurements of serum viral load. J Hepat, Vol. 37, No.1: 61and 63.

56) Samuel C., 2001-Antiviral action of Interferon. J Clin Microbiol Rev, Vol. 14, No. 4: 778.

57) Hanley J and Haydon G., 2001- The biology of Interferon alpha and the clinical significance of anti-Interferon antibodies**.** J Leuk. Lymphoma, Vol. 29: 257.

58) Enomoto M ., Nishiguchi S., Kohmoto M., Tamori A., Habu D.,et al ., 2004-Effects of ribavirin combined with interferon-alpha 2b on viral kinetics during first 12 weeks of treatment in patients with hepatitis C virus genotype 1 and high baseline viral loads. J Viral Hepat, Vol.11: 448.

59)Alter HJ., Majewski D., Rosser M., Thompson A., Mitchell P., Smith T., et al., 1991-The use of a recombinant immunoblot assay in the interpretation of anti-hepatitis C virus reactivity among prospectively followed patients, implicated donors, and random donors. J Transfusion, Vol. 31, No. 8:771.

60) Legrand-Abravanel F., Sandres-Saune K., Barange K., Alric L., Moreau G.,et al., 2004-Hepatitis C virus genotype 5: Epidemiological characteristics and sensitivity to combination therapy with interferon-alpha plus ribavirin. J Infect Dis, Vol. 189: 1397.

61) Haslett C., Chilvers E., Boon N., and Colledge N., 2000-Principles and practice of medicine. Churchill Livingstone, 19th edition. P 864-865.

62) Tortora, Funke, Case, 2004-Microbiology: an introduction. Benjamin Cummings, 8th ed.

63) Lake-Bakaar G., 2003- Current and future therapy for chronic hepatitis C virus liver disease. J Curr. Drug Targets, Vol. 3: 247and 249.

64) Germer, J. J., Majewski D., Rosser M., Thompson A., Mitchell P., Smith T., et al., 2003-Evaluation of the TRUGENE HCV 5'NC Genotyping Kit with the New GeneLibrarian Module 3.1.2 for Genotyping of Hepatitis C

Virus from Clinical Specimens. J Clin. Microbiol, Vol. 41, No. 10 : 4855-4857

65) Saracco G., Olivero A., Ciancio A., Carenzi S., and Rizzetto M., 2003-Therapy of chronic hepatitis C: A Critical Review, J Curr. Drug Targets, Vol. 3, No 1: 29.

66) The Roche Vademecum: www.Roche-Arabia. Com (11/06/2005).

67) Shemer-Avni Y., El-astal Z., Kemper O., El-najjar K., Yaari A., et al., 1998-Hepatitis C virus Infection and Genotypes in Southern Israel and The Gaza Strip. J Med. Virol, Vol. 56:230.

68) Torres MC., Pereira LM., Ximenes R., Araujo AS., Secaf M., et al., 2003-Hepatitis C virus infection in a Brazilian population with sickle-cell anemia, Braz. J Med. Biol. Res, Vol. 36, No. 3 : 323.

69) Nishiguchi S., Kuroki T., Ueda T., Fukuda K., Takeda T., et al., 1992-Detection of hepatitis C virus antibody in the absence of viral RNA in patients with autoimmune hepatitis. Ann. Intern. Med, Vol. 116, No. 1: 86.

70) Farci P., Alter HJ., Shimoda S., Govindarajan LC., Cheung JC. et al., 1996-Hepatitis C virus-associated fulminant hepatic failure, N. Engl. J. Med., Vol. 335: 631.

71) EASL International consensus conference on HCV. 1999, J Hepatology, Vol. 30: 956.

72) Michael L., Edward P., and Larry E., 2005-Clinical Chemistry Principles, procedures, Correlations. Lippincott Williams and Wilkins, 5th ed.

73) Haverstick DM., Bullock GC., and Bruns DE., 2004-Genotyping of hepatitis C virus by melting curve analysis: analytical characteristics and performance. Clin. Chem, Vol. 50, No. 12: 2405.

74) Oliveira M.L.A., Sabino B., Paetzold U., Schreier E., Pauli G., et al., 1999-Distribution of HCV genotypes among different exposure categories in Brazil. Braz. J Med. Biol. Res, Vol. 32, No. 3: 279.

75) Friebe P., Lohmann V., Krieger N., and Bartenschlager R., 2001-Sequences in the 5' non translated region of hepatitis C virus required for RNA replication. J Virol, Vol.75, No.24:12048.

76) Stuyver L., 1993-Typing of hepatitis C virus isolates and characterization of new subtypes using a line probe assay. J Gen. Virol, Vol. 74, No. 6:1093.

77) http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi (Last visited on, 1/04/2006)

78) http://www.mbro.ncsu.edu/BioEdit/bioedit.html (last visited on, 8/04/2006)

79) http://www.ebi.ac.uk/clustalw (Last visited on, 01/04/2006)

80) El-Astal Z., 2003- Hepatitis B and C among governmental health providers in Southern Gaza Strip, Palestine. Sahel Med. J, Vol.6, No.4:99.

81) Jaureguiberry S., Grandiere-Perez L., Ansart S., Laklache H., Metivier S., et al., 2005- Acute hepatitis C virus infection after a travel in India. J Travel Med, Vol. 12: 56.

Appendix 1

السادة المحترمون

السلام عليكم ورحمة الله وبركاته

نتوجه إليكم بجزيل الشكر والامتنان، راجين تعاونكم لإنجاح هذه الدراسة العلمية؛ حيث يهدف هذا البحث لتحديد الطراز الجيني لالتهاب الكبد الفيروسي من نوع C في قطاع غزة.

لذا يرجى منكم التفضل بتعبئة الاستبانة. علماً بأن المعلومات التي سنحصل عليها سوف تحظى بسرية تامة.

شاكرين حسن تعاونكم

الباحثة

1- الاسم (اختياري).....

2- العمر.....

3- الجنس: ☐ ذكر ☐ أنثى

4- عنوان السكن:

☐ محافظات الشمال ☐ محافظات الوسطى ☐ محافظات الجنوب

5- الحالة الاجتماعية:

☐ أعزب ☐ متزوج ☐ غير ذلك

6-عدد سنوات الدراسة.....

7- تاريخ اكتشاف الإصابة بالمرض: .....

8- هل سبق لك وأن سافرت إلى الخارج:

☐ نعم ☐ لا

9- إذا كانت الإجابة على السؤال السابق بنعم فالرجاء تحديد تاريخ السفر:

☐ قبل الإصابة بالمرض ☐ بعد الإصابة بالمرض ☐ لا أعلم

10- إذا كانت الإجابة على السؤال رقم (8) بنعم فالرجاء تحديد اسم البلد التي سافرت إليها:

.....

11- إذا كانت الإجابة على السؤال رقم (8) بنعم فالرجاء تحديد هدف السفر:

☐ للعلاج ☐ لسبب آخر

12-هل أنت تعاني من الفشل الكلوي

☐ نعم ☐ لا

13- إذا كانت الإجابة على السؤال السابق بنعم فالرجاء تحديد ما يلي:

☐ قبل الإصابة بالمرض ☐ بعد الإصابة بالمرض ☐ لا أعلم

14- هل سبق لك أن نقل لك دم أو أحد مكوناته؟

☐ نعم ☐ لا

16- إذا كانت الإجابة على السؤال السابق بنعم فالرجاء تحديد ما يلي:

☐ قبل الإصابة بالمرض ☐ بعد الإصابة بالمرض ☐ لا أعلم

17- إذا كانت الإجابة على السؤال رقم (13) بنعم فالرجاء تحديد اسم البلد التي تم فيها:

.....

18- هل سبق لك أن أجريت لك عملية جراحية؟

☐ نعم ☐ لا

19- إذا كانت الإجابة على السؤال السابق بنعم فالرجاء تحديد ما يلي:

☐ قبل الإصابة بالمرض ☐ بعد الإصابة بالمرض ☐ لا أعلم

20- إذا كانت الإجابة على السؤال رقم (16) بنعم فالرجاء تحديد اسم البلد التي تم فيها :

.....

21- هل سبق أن أجري لك فحص PCR ؟

☐ نعم ☐ لا