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Epidemiology, Molecular genotyping and risk factors of HAV infections in the West Bank, Palestine

Hayah Samih Ahmad Qaraqe

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Epidemiology, Molecular genotyping and risk factors of HAV infections in the West Bank, Palestine

Prepared by:
Hayah Samih Ahmad Qaraqe

B. Sc. Medical Technology - Al-Quds University ,Palestine

Supervisor: Dr. Rasmi Abu Helu

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Thesis Approval

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Prepared By: Hayah Samih Ahmad Qaraqe

Registration Number: 21310227

Supervisor: Dr. Rasmi Abu Helu.

Master thesis Submitted and Accepted, Date: 17 / 12 / 2017

The names and signatures of the examining committee members are as follows:

1- Head of Committee: Dr. Rasmi Abu Helu Signature: .....

2- Internal Examiner: Dr. Sameer A.Barghouthi Signature : .....

3- External Examiner: Dr. Kamal Dumaidi Signature : .....

Jerusalem – Palestine

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Dedication

I dedicate this work to my mother and father,

to my dear husband Khalid,

to my Lovely Kids; Talya and Ali,

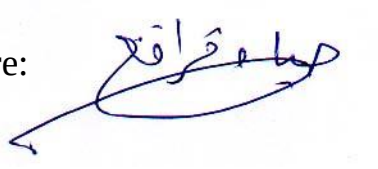
to my sisters and my brothers,

to all teachers,

to my friends.

Declaration

I certify that this thesis submitted for the degree of Master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signature: 

Name: Hayah Samih Ahmad Qaraqe

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Abstract:

Background

Hepatitis A virus (HAV) is a food and water-borne virus causing clinical (mainly hepatitis) and subclinical disease in human. diagnosis and genotyping of HAV infection is helpful for proper treatment and epidemiology surveillance.

The aim of the present study was to determine the molecular genotyping and the risk factors of HAV infection in the West Bank, Palestine using serology and Molecular assay.

Method:

In this study, 272 HAV suspected cases were analyzed by ELISA for the presence of antibodies to HAV. RNA was extracted from 110 HAV IgM positive human sera. Samples found positive by RT-PCR using primers targeting the VP1/VP2A junction and VP1/VP3 capsid region of HAV, were subjected to sequencing and phylogenetic analyses.

Results

IgM type antibodies to HAV were detected in 272 patients ;136 sample as the control group were negative for HAV IgM, and 136 cases was positive for IgM,75.74% of them were student, differed in age groups and was higher in the age group ≤10years. Phylogenetic analysis showed that the majority of HAV strains detected in this study belong to the “HAV 1B” cluster.

Conclusions

The results indicate that molecular studies determining the HAV genotype variation in Palestine are timely and warranted. The majority of IgM positive cases in ≤10 year-old patients. Sub-genotype IB is the most prevalent genotype in Palestine.

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Abbreviations Used:

AL	Lysis Buffer
ALT	Alanine amino transferase Enzyme
AST	Aspartate amino transferase Enzyme
AVE	Elution Buffer
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
HAV	Hepatitis A virus
IG	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IRES	internal ribosome entry site
NCR	non-coding region
ORF	open reading frame
PCR	Polymerase Chain Reaction
QIAmp	Qiagen DNA Kit
RT	Reverse Transcription
RT-PCR	Reverse transcription polymerase chain reaction
5'-UTR	5'-untranslated region
3′UTR	3′-untranslated region
VP	virion protein

Introduction:

Hepatitis A virus (HAV) genome is a positive single-stranded RNA genome approximately 7.5 kb long. The HAV genome composed of three regions; 5' non-translated region (5' UTR), open reading frame (ORF) and 3' untranslated region (3; UTR) with poly-A tail (Hussain et al.,2011).Hepatitis A virus (HAV) is endemic worldwide, and the most causative agent of viral hepatitis, with around half of the cases of hepatitis diagnosed worldwide. The HAV endemicity level for a population is defined by the results of age-seroprevalence surveys; a systematic review on the global prevalence of HAV infection was recently published by the World Health Organization (WHO,2010). In addition, recently it has been reported as one of the most important human food-borne pathogens and is the cause of most outbreaks reported in the Western world. More than 1.4 million new HAV infections are reported each year. Most of the world's population is still at moderate-to-high risk of hepatitis A virus (HAV) infection. Areas of the world can be characterized as having high, intermediate and low endemicity for hepatitis A. In less developed countries with very poor sanitary and hygienic conditions, HAV infection is highly endemic and most persons become infected in early childhood. Because infection occurs at an early age when the disease is often asymptomatic, reported rates of the disease in these areas are relatively low and outbreaks are not common. Areas of high endemicity include most of Africa, Asia and Central and South America. Conditions which contribute to the propagation of the virus among young children in these areas include household crowding, poor levels of sanitation and inadequate water supplies (WHO,2010). In developing countries and some regions of developed countries, which include Eastern Europe, parts of Africa, Asia and America, sanitary and hygienic conditions vary and some children avoid infection during early childhood. Peak rates of infection commonly occur in later childhood or adolescence. Paradoxically, since HAV transmission occurs in these

areas in older age groups, reported rates of hepatitis A can be higher than in less developed countries where HAV transmission is more highly endemic. In most developed countries, such as North America, Western Europe, Australia and Japan, sanitation and hygienic conditions are generally good and infection rates in children are generally low. Peak rates of infection and reported disease tend to be among adolescents and young adults. In these areas, large community-wide outbreaks with extended person-to-person transmission can still contribute significantly to the burden of hepatitis A disease. In addition, occasional outbreaks in child care centers or residential institutions and food borne or waterborne epidemics can occur. In some countries with very low prevalence (e.g. Northern Europe), disease predominates among specific adult risk groups: travelers to countries where hepatitis A is endemic; intravenous drug users; and men with a history of homosexual behavior. The prevalence of anti-HAV increases gradually with age, primarily reflecting declining incidence, changing endemicity and a resultant lower childhood infection rate over time(WHO,2010).At the same time, as the countries around the world developed economically, the prevalence of HAV infection will probably fall, but paradoxically hepatitis A illness will continue considered a public health problem because the severity of symptomatic illness with HAV infection are age-related (2–4years) (Victor et al., 2006).Finally, HAV is endemic in most of the middle east countries with different prevalence rate. In Palestine, (1,031) cases was reported in 2014 with an incidence rate 22.7 per 100,000 of population; 171 cases in West Bank with an incidence rate 6.1 per 100,000 of population. (MOH,2014).

In 2015, the reported cases were 1,397 with an incidence rate of 29.8 per 100,000 of population; 481 cases were in West Bank with an incidence rate of 16.8 per 100,000 of population. (MoH,2015)

In 2016, there was a decrease in the number of cases reported, 780 cases of viral hepatitis A were reported with an incidence rate of 17.1 per 100,000 populations, of which 313 cases in West Bank with an incidence rate of 11.7 per 100,000 populations.

The mortality rate changes according to the age, while severity of the infection is higher in adults than children. It is not possible to distinguish HAV strains by serotyping, but seven genotypes can be differentiated with molecular methods.(Kokkinos et al., 2010; Dinc et al., 2012).

However, the risk factors and the genotypes of the endemic HAV are not investigated yet. Therefore, the aims of the present study were to investigate the different genotypes as well as the risk factors of HAV infections in the West Bank, Palestine during the period of (2014-2016).

This study will allow the investigation of a molecular epidemiology of HAV from patient with acute hepatitis A, will also determine the demographic distribution of HAV, optimizing RT-PCR for direct diagnosis and typing of HAV from clinical sample and will investigate the most predominant genotype of HAV that circulates in Palestine as well as to study molecular characterization.

Chapter One:

Literature Review

1.1Virology:

Hepatitis A virus (HAV) is a *Hepatovirus*, member of the *Picornaviridae* family, is a non-enveloped (naked), linear, positive single stranded RNA virus of an icosahedral symmetry measuring 27-32 nm in diameter (Figure1). The infectious particle consists of capsid protein and RNA genome and it has lifelong immunity after natural infection because has a single antigenic serotype (Anderson et al., 1988). The virus is thermostable and acid-resistant. Previously, HAV was thought to be an enterovirus; in 1991, it was subclassified into its own unique genus hepatovirus. (Franck et al., 1991). Only one human HAV serotype has been identified. HAV strains that are isolated from various parts of the world constitute a single serotype but variations in the nucleotide sequences are conceivable in the genome district. VP1-2A allows the classification of HAV into seven different

genotypes (genotypes I, II, III and VII); which are associated with human infections, whereas genotypes IV, V and VI cause infections in simians, Subtype IA appears to be responsible for the majority of hepatitis A cases worldwide, but subtype IB viruses have been found in the Mediterranean region (Gharbi-Khelifi et al., 2012).

The human genotypes that have been identified are stable and can be used to trace transmission. HAV vaccine prepared from any human HAV genotype will provide protection against infection by all strains (Robertson et al., 1992). After oral inoculation, HAV is thought to be transported across the intestinal epithelium by a vectorial transport process that is poorly understood, and is taken up by hepatocytes. Specific receptors are involved remains uncertain, although a novel glycoprotein has been identified as a cellular receptor for HAV in one cell line (Shavrina Asher LV et al.,1995). The liver is the only target organ of injury; HAV genomic replication occurs exclusively in the cytoplasm of the infected hepatocyte by a mechanism involving an RNA-dependent RNA polymerase. From the liver, HAV is transported through the biliary tree to the intestine; its resistance to inactivation by bile and intestinal proteolytic enzymes allows it to be shed in the feces facilitating fecal-oral transmission. (Kaplan G et al.,1996).

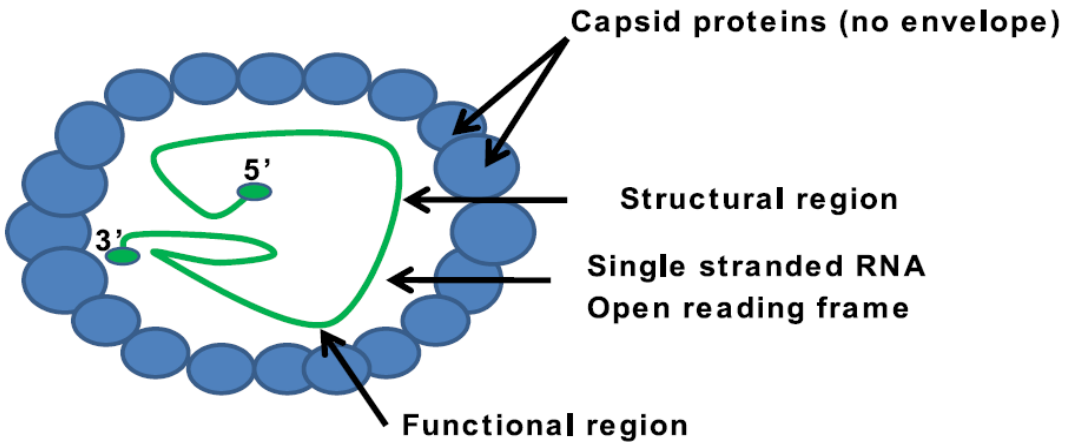

Fig. 1. The internal structure of hepatitis A virus showing capsid proteins and envelopes, structural region, positive single stranded RNA (open reading frame) and functional region. (Adapted from: Anderson et al., 1988).

1.2Genome composition and Organization:

Hepatitis A virus (HAV) is known as a positive sense, single-stranded RNA genome with approximately 7.5 kb long and has a large open reading frame that divided into three functional areas (P1-P3). The P1region encodes capsid polypeptides VP1-VP3 and putative VP4. The P2 and P3 areas encode nonstructural proteins which are essential for infection and replication (Hussain et al.,2011). In addition, HAV has a 5'nontranslated region that comprises approximately 10% of the HAV genome and a 3' untranslated region with poly-A tail. After cleavage by proteases, the single polyprotein of HAV yields three major protein groups. While P1 encodes capsid proteins VP1- VP3, P2 and P3 encode non-structural proteins related to viral replication, including proteases and polymerase (Figure 2). (Hussain et al., 2011).

HAV genomic heterogeneity has been revealed based on different genome regions, including those encoding for the virion protein VP3 C-terminus, the VP1 N-terminus and the VP1/2A junction (168bp), which is seems to be the most factor utilizer for HAV genotypes (Gharbi-Khelifi et al., 2012).

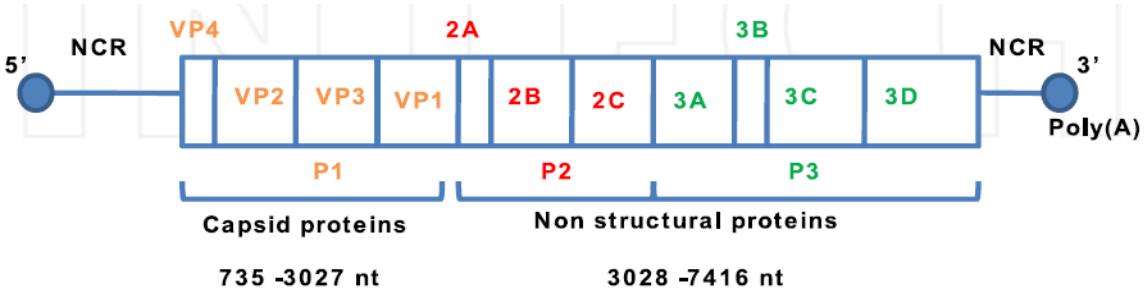

Fig. 2. Genomic structure of hepatitis A virus: HAV genome is divided into a 5' un-coding region (5' UCR), a giant open reading frame, and un-coding region (3' UCR). The coding region is subdivided into regions P1, P2 and P3. (Adapted from: Hussain et al.,2011)

Structural proteins are cleaved by viral protease 3C that is encoded by the P3 region and is responsible for most cleavages within the polyprotein. Nonstructural proteins are processed

in the P2 and P3 regions and are required for RNA synthesis and virion assembly. The P3 segment contains the 3A, 3B, 3C and 3D proteins. (MinKyung et al.,2002).

The 5′ NTR which is the conserve region of the viral genome, has a covalently linked virus-specific protein (VPg) rather than a cap structure. This region comprises approximately of 735 to740 nucleotides in length, uncapped, covalently-linked to the genome, contains an internal ribosomal entry site (IRES) and plays an important role in the initiation of translation. (Vaughan et al., 2014; Brown, Day, Jansen& Lemon, 1991). The 3′UTR of 40 to 80 nucleotides, has a poly(A) tract and translation terminator sequence(Vaughan et al., 2014; Brown, et al., 1991).The 3′-UTR of 40 to 80 nucleotides, has a poly(A) tract and translation terminator sequence (Nainan et al., 2006).

Numerous regions on the HAV genome have been used for genotyping analysis by sequence analysis including, the C terminus of the VP3, N terminus of the VP1, VP1-2A junction, VP1-2B region, entire VP1, and the VP3-2B region (Dinc et al., 2012).

1.3 Life cycle and Replication

HAV is most commonly contracted via feco-oral route. (Hollinger and Emerson, 2007; Sherlock et al., 2008; Spradling et al., 2009). After replication in the oropharynx, salivary glands and gastrointestinal tract (the primary sites of virus replication), the virus is transported to the major site of replication, the liver, where it is subsequently released from liver cells into the bile and then excreted in the feces (Cohen et al., 1989; Lees, 2000; Cuthbert, 2001; Hollinger and Emerson, 2007). Figure 5 shows the life cycles of HAV.

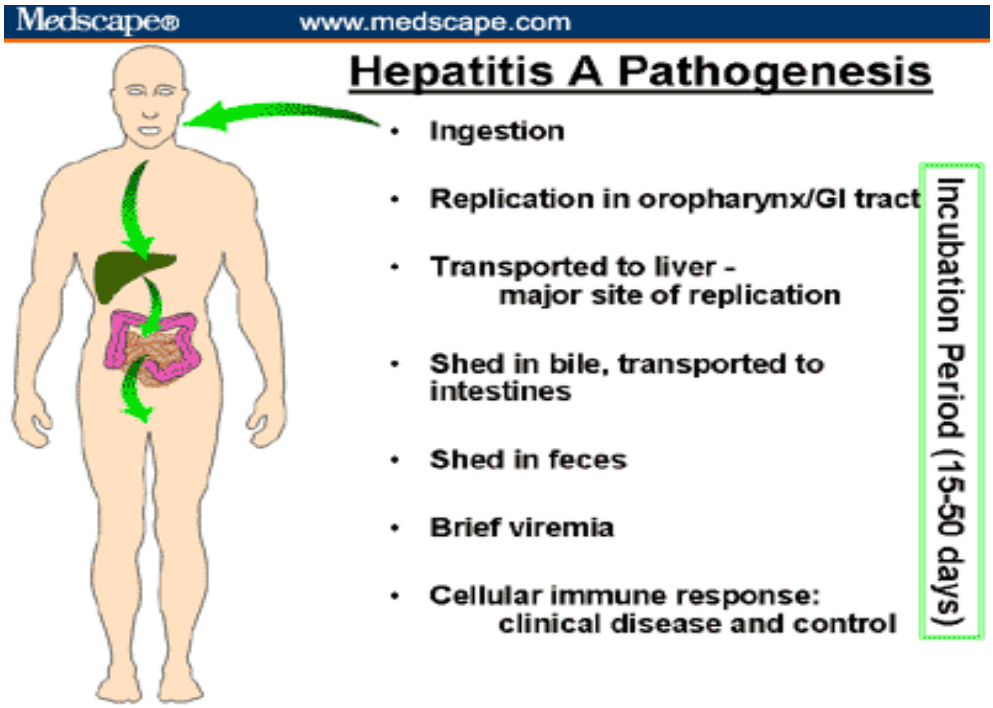

Figure3. Lifecycle of Hepatitis A virus showing fecal-oral route. (Adapted from source: http://pathport.vbi.vt.edu/pathinfo/pathogens/HAV.html).

The highest level of viral particles is detected in the stool during the initial acute 2-weeks period followed by the onset of jaundice, and then levels decrease once jaundice is apparent (Tassopoulos et al., 1986). Children can shed the virus for long periods up to 10 weeks after the onset of clinical illness, whereas infants can shed HAV for up to 6 months (Robertson et al., 2000; Rosenblum et al., 1991). There is no evidence of chronic shedding of HAV in feces; however, recurrent shedding occurs in persons who have relapsing illness (Sjogren et al., 1987). Viremia is also found at the same time when HAV particles are shedded in fecal during the incubation period (Hollinger and Emerson, 2007). HAV is similar to other picornaviruses in its preference to cytoplasmic replication (Collier and John, 2006). Clinical symptoms or an immunologic response does not occur during the incubation period (Nainan et al., 2006). Though the complete viral life cycle is not fully understood, a general overview of the process can be described based on prior studies in cultured monkey kidney and human cells (Feigelstock et al., 1998; Kaplan et al., 1996). The initial step of infection is when the virus has gained entry into the blood of an

individual. The virus then circulates through the body to the liver. HAV binds via its envelope heterodinemic glycoprotein to a cellular receptor (HAVrc1), a mucin-like glycoprotein, on hepatocytes (Feigelstock et al., 1998; Kaplan et al., 1996). The binding of HAV and HAVcr1 leads to particle conformational changes and uncoating of the virion (Silberstein et al., 2003). The virion uncoats and the viral RNA is released into the cytoplasm at about 4 h post-infection (Spradling et al., 2009). After releasing into the cytoplasm, the uncoated RNA genome is translated through an IRES-dependent mechanism requiring cellular initiation factor to function (Borman and Katherine, 1997). To replicate its genome, the positive strand RNA of HAV serves as a template for negative strand RNA synthesis by the RNA-dependent RNA polymerase 3D. Then, this negative strand intermediate acts as a template for the synthesis of several positive strand RNA molecules, which can be translated into proteins, replicated, or packaged into new virion (Spradling et al., 2009).

1.4Transmission and molecular epidemiology:

HAV infection is a widespread infection; it is the cause of approximately one-half of all reported cases of viral hepatitis in the United States. In 2009, it was estimated that more than 21,000 cases of hepatitis A occurred in the United States, and 1.4 million cases occurred worldwide each year (Keefe, 2006). Africa, Latin America, the middle East, and parts of Asia are areas of high endemicity where the majority of infections occur in early childhood and show high prevalence of anti-HAV up to 90% in adults (Spradling et al., 2009).

The variable age distribution among hepatitis A patients in developing and developed countries is a consequence of differing standards of hygiene and sanitation.

In developing countries with poor sanitary conditions and hygienic practices, most children (90%) have been infected with the hepatitis A virus before the age of 10 years. Those

infected in childhood do not experience any noticeable symptoms. Epidemics are uncommon because older children and adults are generally immune. Symptomatic disease rates in these areas are low and outbreaks are rare, countries with transitional economies, and regions where sanitary conditions are variable, children often escape infection in early childhood and reach adulthood without immunity. Improved economic and sanitary conditions may lead to accumulation of adults who have never been infected and who have no immunity. This higher susceptibility in older age groups may lead to higher disease rates and large outbreaks can occur in these communities, countries with good sanitary and hygienic conditions, infection rates are low (WHO,2017).

The greater part of the world's population is still at moderate-to-high risk of hepatitis A virus (HAV) infection. At the same time, as the countries around the world developed economically, the prevalence of HAV infection will probably fall, but paradoxically hepatitis A illness will still consider a greater public health problem. This is because the likelihood and severity of symptomatic illness with HAV infection are age-related (2-4 years)(Victor et al., 2006).HAV infection does not lead to chronic or persistent hepatitis.(Hirai et al., 2016; Lima et al., 2014). HAV molecular epidemiology is important to understand the strains that circulate in various geographical regions and trace the source of contamination in an outbreak situation (Singh et al., 2015). The most reported risk factor of HAV infection ingestion of infected food or water or person-to-person contact, low educational level, Low income, traveling to endemic areas, hemophiliacs and contaminated food and drink usage, crowding and lack of access to safe drinking water and sanitation facilities. Furthermore, homosexuality (men have sex with men) and drug addicts had been reported as HAV risk factors (Jacobsen & Koopman, 2005;Munne et al., 2007; Hutin et al., 2000; D'Andrea et al., 2015). HAV infection is a worldwide distributed, although its endemicity varies significantly at both national, regional and international levels. It is not

possible to distinguish HAV strains by serotyping, but seven genotypes can be differentiated with molecular method (Lemon,1997).

The HAV incidence is thought to be much higher than reported since not all infected individuals were seek medical intervention and thus are not reported. Lower incidence rate and fewer HAV cases are found after improvements in sanitation and living conditions, predominantly among all populations in developed countries and those with higher socio-economic status in developing countries (CDC, 1999).

Based on serological analyses, a given region can be categorized as having high, intermediate and low endemicity: in areas with high endemicity, nearly 100% of the adolescent and adult population is seropositive, that is with the infection having been acquired during childhood. Areas with intermediate endemicity are characterized by a shift in the age of seropositive individuals, from children to adolescents and adults. To date, the severity of the disease increases progressively with age, whereas infections during childhood are often asymptomatic, occurrence during adulthood is symptomatic and associated with a mortality rate of up to 2% in industrialized countries among patients >40 years (Koff, 1998).

Based on Molecular epidemiology The worldwide genotype distribution showed genotype I and III comprise the vast majority of human strains within the studied population .Sub-genotype IA comprises the majority of the human strains studied and constitutes major virus population in North and South America, China, Japan, Russia and Thailand. The sub-genotype IB contains strains from Jordan, North Africa, Australia, Europe, Japan and South America. Most of the remaining human HAV strains segregate into genotype III that is further divided into two sub-genotypes, IIIA, and IIIB . The sub-genotype IIIA have been subsequently identified in specimens collected from humans with hepatitis A in India,

The IIIB subgenotype is responsible for cases of HAV infection in Japan and Denmark (Cohen, 1987; Jansen, 1990; Robertson, 1992).

1.5 Pathogenesis:

Virus-induced cytopathology may not be responsible for the pathologic changes seen in HAV infection as liver disease may result primarily from immune mechanisms. Antigen-specific T-lymphocytes are responsible for the destruction of infected hepatocytes (Wang C-H et al.,1996).

Cell-mediated immune mechanisms are thought to bring about the necroinflammatory lesions of hepatitis A; CD8 T lymphocytes and natural killer cells have been implicated. Hepatocyte death may also occur by apoptosis. In the acute phase of hepatitis A, a high level of IgM and IgG, the VP1 protein, which is a major HAV capsid polypeptide, has been identified; in serum collected during the early and late convalescence period. Antibodies to VP3 and VP1 are also present. Circulating anti-HAV may interfere with the intrahepatic spread of HAV to contiguous susceptible cells and limit the development of secondary viremia. Non-secretory IgG antibody to HAV is present indefinitely in serum after infection. This type of antibody seems to be the means by which the lifelong immunity is maintained (Wang C-H et al.,1996). Increased levels of interferon have been detected in the serum of HAV-infected patients and are presumably responsible for the reduction in virus burden seen in patients following the onset of clinical disease and symptoms (Hollinger, 1995).

Patients with acute viral hepatitis A develop features of cholestasis. Confluent hepatic necrosis may lead to fulminant hepatitis and death in 30 -60% of cases. Death appears to be inevitable when necrosis involves more than 65 - 80% of the total hepatocyte fraction.

In patients who survive an episode of acute fulminant hepatic failure, neither functional nor pathologic sequelae are common, despite the widespread necrosis. During the recovery stage, cell regeneration is prominent. The damaged hepatic tissue is usually restored within 8 to 12 weeks (Lemon, 1997).

1.6 Clinical sign and symptom:

Hepatitis A infection is mainly asymptomatic among children less than 6 years old and symptomatic among older children and adults. After an average incubation period of 28 days (range, 15 to 50 days), most HAV-infected persons developed nonspecific signs and symptoms followed by gastrointestinal symptoms. These, include fever, malaise, anorexia, nausea, abdominal discomfort, dark urine and jaundice. About 15 to 20% of the patients may have prolonged or relapsing disease lasting up to 6 months (Figure 4). HAV infections has also been detected as long as 6 to 12 months after infection (Nainan et al., 2006;Melhem et al., 2015). Exposure of non-immune adolescents and adults may result in severe clinical disease like fulminant hepatic failure (Hussain et al., 2011).

Fulminant hepatitis is a rare complication of hepatitis A. The risk of acute liver failure ranges from 0.015 to 0.5%, and the highest rates occur among young children and older adults with underlying chronic liver disease (Nainan et al., 2006).

The fulminant hepatitis A causes necrosis of the liver that followed by destruction of hepatic synthetic processes, excretory functions and detoxifying mechanism. These complications occur during the first 4-6 weeks of illness which are characterized by rapid onset of high fever, marked abdominal pain, vomiting and jaundice followed by development of hepatic encephalopathy associated with deep coma and seizures. Mortality increased with rising age and survival being rare over the age 45 years. Clinical signs that indicating liver failure include a rapid decrease in the size of the liver, prolongation of the

prothrombin time and decrease in the aminotransferase level and the bilirubin level and continues to rise (Hussain et al., 2011).

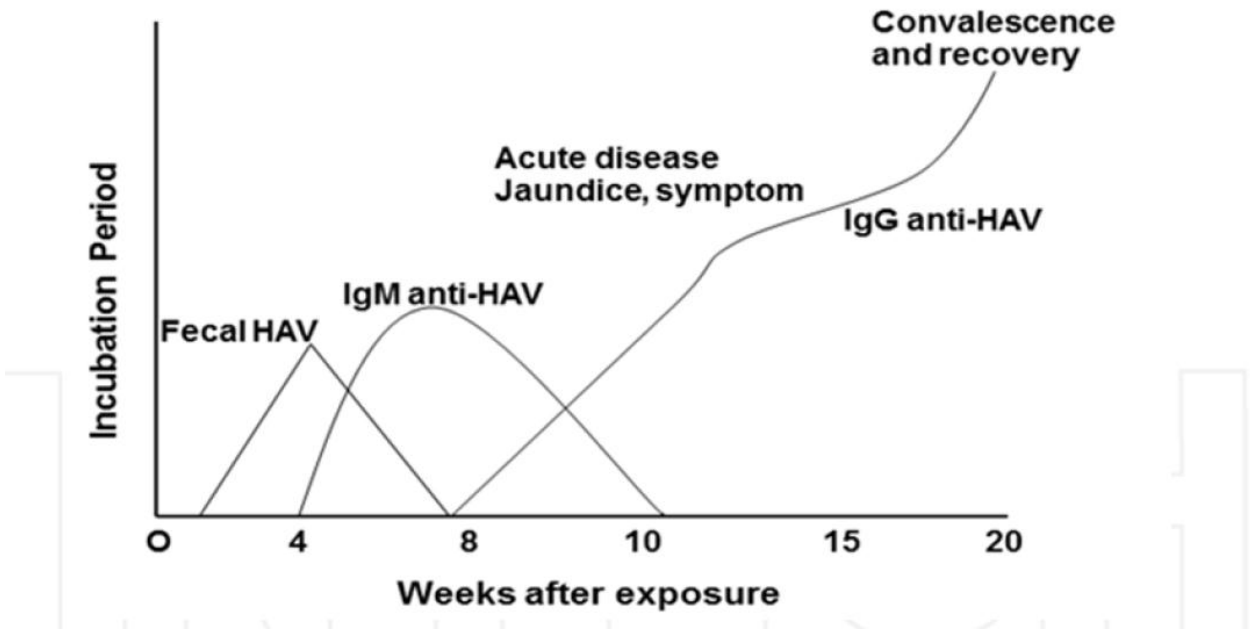

Fig. 4. The serological course of hepatitis A virus. The virus can be detected in the feces up to 2 weeks before the appearance of the jaundice and up to 2 weeks afterwards. (Adapted From Lemon, 1997)

1.7 Mortality rate:

The vast majority of hepatitis A patients make a full recovery and fatality rate is low. The estimated mortality rate is 0.1% for children less than 15 years old, 0.3% for adults ages 15-39 and 2.1% for adults over 40 years (Hollinger, 1996; Debray, 1997). The acute HAV super infections with chronic liver disease is also associated with severity and high mortality rate (Keffe, 1995; Vento, 1998).

1.8 Treatment:

Specific treatment is not available for acute hepatitis A virus infection. As in most cases the infection is self-limiting and is followed by complete recovery without chronic sequelae, and no specific interventions are required. Complete recovery from symptoms following infection may be slow and take several weeks or months. Therapy is aimed at maintaining comfort and adequate nutritional balance, including replacement of fluids that are lost from vomiting and diarrhea (André, 1995).

1.9 Antigenicity:

HAV has only one known serotype, different viral strains show similar reactivity to monoclonal anti-HAV antibodies. Antigens of the intact virion are conformational and different from those of isolated proteins. Antibodies to purified capsid proteins or to synthetic peptides have weak or no detectable neutralizing activity is neutralized by both anti-HAV IgG and anti-HAV IgM. No serologic or hybridizing cross reactivity between HAV and other viral hepatitis agents, including hepatitis E virus (HEV), have been observed. The nonstructural proteins of HAV are also immunogenic during natural and experimental infections (Hollinger, 1996; Lemon, 1997)

1.10 Diagnosis:

Acute hepatitis due to HAV cannot be distinguished from that due to the other hepatitis viruses, serologic tests are necessary for a virus-specific diagnosis.

Diagnosis of hepatitis is made by biochemical assessment of liver function (laboratory evaluation of: urine bilirubin and urobilinogen, total and direct serum bilirubin, Alanine aminotransferase enzyme (ALT) and/or Aspartate amino transferase enzyme (AST), alkaline phosphatase, prothrombin time, total protein, serum albumin, HAV IgG, IgA, IgM, complete blood count). The classical routine diagnosis of acute hepatitis A is made

by finding anti-HAV IgM in the serum of patients. A second option is the detection of virus and/or antigen in the blood and/or the feces.

HAV antibodies can be detected by using commercially immunoassay kits (Koff, 1998). These assays for anti-HAV IgM and total anti-HAV (IgM and IgG) for assessment of HAV status are not influenced by the passive administration of IG, because the prophylactic doses are below detection level. At the onset of disease, the presence of HAV IgG is always accompanied by the presence of IgM. As IgG anti-HAV persists lifelong after acute infection, detection of IgG anti-HAV alone indicates past infection. However, commercially available assays detect total anti-HAV (both IgG and IgM antibodies). The presence of total anti-HAV and the absence of IgM anti-HAV can be used to differentiate acute and previous infections (Lemon, 1997).

For molecular diagnosis Nucleic acid detection techniques are more sensitive than immunoassays for viral antigen to detect HAV in samples of different origins (e.g., clinical specimens, environmental samples, or food). HAV has been detected with techniques such as restriction fragment length polymorphism, single-strand conformational polymorphism, Southern blotting, nucleic acid sequencing-based amplification, nucleic acid hybridization, and reverse transcription-PCR (RT-PCR) and antigen capture RT-PCR. Amplification of viral RNA by RT-PCR is currently the most sensitive and widely used method for detection of HAV RNA (Nainan et al., 2006).

1.11 Prevention Methods and control:

Prevention methods include improvement of personal hygiene and sanitation are critical control of measures for HAV. Control of water quality and management of food are also important to prevent outbreaks. Prevention of clinical manifestation of Hepatitis A through administration of immune globulin is used mainly for close personal contacts of patients

with hepatitis A and for those exposed to contaminated food. Active immune-prophylaxis with formalin- inactivated hepatitis A vaccine is highly effective in the prevention of hepatitis A. The epidemiology of Hepatitis A varies in different part of the world. Hepatitis A vaccine is most effective in preventing illness post-exposure if used within 14 days of exposure, particularly if used in conjunction with passive immunization with immune globulin (IG). It is recommended for certain susceptible contacts following potential exposure to HAV. Therefore, vaccination strategies have to be implemented based on the prevalence of the HAV endemicity (CDC, 2003)

1.12 Objectives:

Main Objective: to investigate the HAV molecular epidemiology among acute hepatitis patients in comparison with control group in the West Bank, Palestine during the period of (2014-2016).

Specific Objective:

1. To determine HAV risk factors using molecular assays
2. To determine the most risk factors of HAV infection
3. To study the molecular characterization of the HAV isolates in comparison to isolates from the neighboring countries and others
4. Develop recommendation based on this study

Chapter Two:

Materials and Methods:

2.1 Serum collection:

Study design: cross-section study design

Study period: January2014- December2016

Study participants: a convince 136 IgM HAV cases were obtained during the period of 2014-2016. These samples were obtained from the Palestinian central public health Laboratories in which all the HAV susceptible cases referred. An equal 136 individuals during the same period which showed IgM HAV negative result were selected as a control group.

The age of HAV cases was range from 1 to 40 years. The cases originated from Alkhalil, Tubas, Jenin, Salfit, Ramallah, Bethlehem, Jericho, Al-Quds, Qalqelya and Tulkarem.

A total of 0.5 ml serum sample from each cases were liquated into two sterile Eppendorf tubes (one for HAV IgM and the other for HAV RT-PCR) and stored at -20°C till tested.

Data collection:

The general characteristic, demographic data and the clinical history for each participant were collected from the patient's medical files and by using a baseline questionnaire; the collected data include age, sex, demographic, educational level of the parents, clinical, socioeconomic information, occupation (Appendix A).

2.2 Serological assays:

All the serum samples of the cases and the control group were tested for HAV IgM antibodies at the central Public health laboratories, Palestinian Ministry of Health using commercially IgM capture ELISA (Architect ELISA) with a sensitivity and specificity of >99% according to the manufacturer's instructions.

2.3 Molecular assays:

2.3.1 Extraction of viral RNA:

The HAV viral RNA was extracted from 200µl of serum samples, using a QIAamp MiniElute Virus spin kit for the viral RNA extraction (QIAGEN,) according to the manufacturer's instructions. The final volume of the RNA elute was 35µl. Briefly, 200 µl serum was added to 25 µl protease k into sterile eppendorff tube. Then 200 µl of AL buffer (lysis buffer containing 28 µg/ml of carrier RNA) was added to the mixture. After vortex mixing, the mixture was incubated for 15 minutes at 56 ^{o}C. Then, 250 µl of absolute ethanol was added to the mixture and mix well and the lysate was incubated for 5 minutes at room temperature (15–25°C). lysate were transfer into the QIAamp MinElute column and centrifuge for 1 minute at 8000rpm. Then the QIAamp MinElute column was washed twice using washing solution provided in the extraction kit. Then, 500 µl of ethanol (96–100%) was added to MinElute column and centrifuge at 8000 rpm for 1 minute. finally after removing the ethanol, the RNA was eluted into sterile eppendorff tube with 35 ul of

Buffer AVE (is RNase-free water that contains 0.04% sodium azide to prevent microbial growth and subsequent contamination with RNases) provided in the kit. The extracted RNA was store at -20 ^{o}C until tested by PCR.

2.3.2 Reverse transcription-polymerase chain reaction (RT-PCR) for detection of HAV genome:

The HAV RNA of 110 available serum samples from the IgM HAV positive cases was amplified using two primers targeting the VP3/VP1 region of the viral genome described previously by Lee et al., (2014). Briefly, the synthesis of the cDNA and the first round amplification of the RT-PCR was carried out in 25µl- reaction mixture containing 4 µl viral RNA extraction, 10 U Reverse transcriptase (AMV), 10 pmol of each the forward and the reverse primers: (HAV1; 5' -GCTCCTCTTTATCATGCTATGGAT-3' and rHAV2; 5'-CAGGAAATGTCTCAGGTACTTTC-3') and 12.5 µl of PCR Reddy master mix (Thermo Scientific). The PCR protocol was: Reaction for reverse transcription was performed for 40 min at 48°C, followed by denaturation for 4 min at 95°C; 30 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 55°C, and extension for 30 s at 72°C; and a final extension step for 7 min at 72°C. PCR products (6 ul) were loaded onto a 2% agarose gel, electrophoresed, and stained with ethidium bromide for band visualization (expected lengths, 244bp) using the Gel Doc System 2000 (Bio-Rad Laboratories-Segarate, Milan, Italy).

Eighteen of the 68 PCR positive cases were selected for sequence analysis. To be representative, the selection of the 18 sequenced cases was based on the two years of the study, the different geographical distribution and the months of HAV cases.

The PCR amplicon of the 18 PCR positive amplicon was purified and sequence.

HAV identity search was conducted using GenBank Basic Local Alignment Search Tool (BLAST) http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi. A multiple nucleotide alignment of isolates in this study and other HAV published sequences was produced using MEGA6. A phylogenetic tree was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura K. et al.,1993) with 1000 bootstrap 1000 replicates

2.4 Statistical analysis:

A database was set up using the EPI INFO software version 7. The participant demographic and socioeconomic status including gender, age, educational level, occupation, and region were derived from their study questionnaire. For comparison of categorical variables between groups were assessed by using the Chi square test and fisher's exact test. The level of significance adopted for all test was ($P < 0.05$).

2.5 Ethical consideration

A written informed consent was obtained from each patient or their Family after they had been informed about the aim of the study. They were informed that the questionnaire would be administered in privacy, the development of a formal questionnaire ensured that similar data were collected from all participants and it also ensured objectivity during the data collection process. (Appendix A).

Information about the study was given by the research from primary health care centers of Ministry of Health, and also by central Public health laboratories (Appendix B and C).

Chapter Three:

Results:

3.1 Description of the participants:

A total, 272 subjects (136 HAV IgM positive cases and 136 HAV IgM negative) were participated in this study. Of the HAV cases, 82 (60.29%) were males and 54 (39.71%) were females. The general characteristic of the cases and the control group are shown in table 1.

Table3.1: general characteristic of the cases and the control group participated in the study.

Variables	**Participants N (%)**	
Gender	HAV cases group	Control
Male	82 (60.29%)	60 (45.11%)
Female	54 (39.71%)	73 (54.89%)
Age		
≤10	67 (49.28%)	21 (15.54%)
>10 -≤ 20	52 (38.24%)	27 (19.98%)
>20 - ≤ 30	15 (11.05%)	48 (35.55%)
>30 - ≤ 40	2 (1.48%)	20 (14.80%)
≥ 40	0 (0%)	19 (14.06%)
Place of District		
Tulkarem	29(21.32%)	7 (5.26%)
Alkhalil	56 (41.18%)	21(15.79%)
Qalqilia	3 (2.21%)	8 (6.02%)
Tubas	2 (1.47%)	3 (2.26%)
Nablus	9 (6.62%)	9 (6.77%)
Ramallah	6 (4.41%)	57(42.86%)
Ariha	10 (7.35%)	7 (5.26%)
Bethlehem	17(12.50%)	11 (8.27%)
Al-Quds	2(1.47%)	0 %
Salfit	2(1.47%)	0 %
Marital status		

Married	7 (5.14%)	63(46.3%)
Single	129 (94.8%)	67(49.2%)
Divorced	0	1(0.73%)
Level of education		
< Tawjihi	99 (72.79%)	49 (36.03%)
>Tawjihi	37(27.20%)	87 (63.97%)
Income (monthly income in sheikel)		
≤ 1450	12 (8.89%)	10 (7.35%)
>1450	123 (91.11%)	118 (86.76%)
Occupation		
Student	103 (75.74%)	36 (26.67%)
Housewife	5 (3.68%)	28 (20.74%)
Worker	9 (6.62%)	34 (25.19%)
Clerical worker	1 (0.74%)	9 (6.67%)
None	18 (13.24%)	28 (20.47%)

N: number of su

The participants (the cases and the control group) were recruited from all the Palestinian districts see Figure 5.

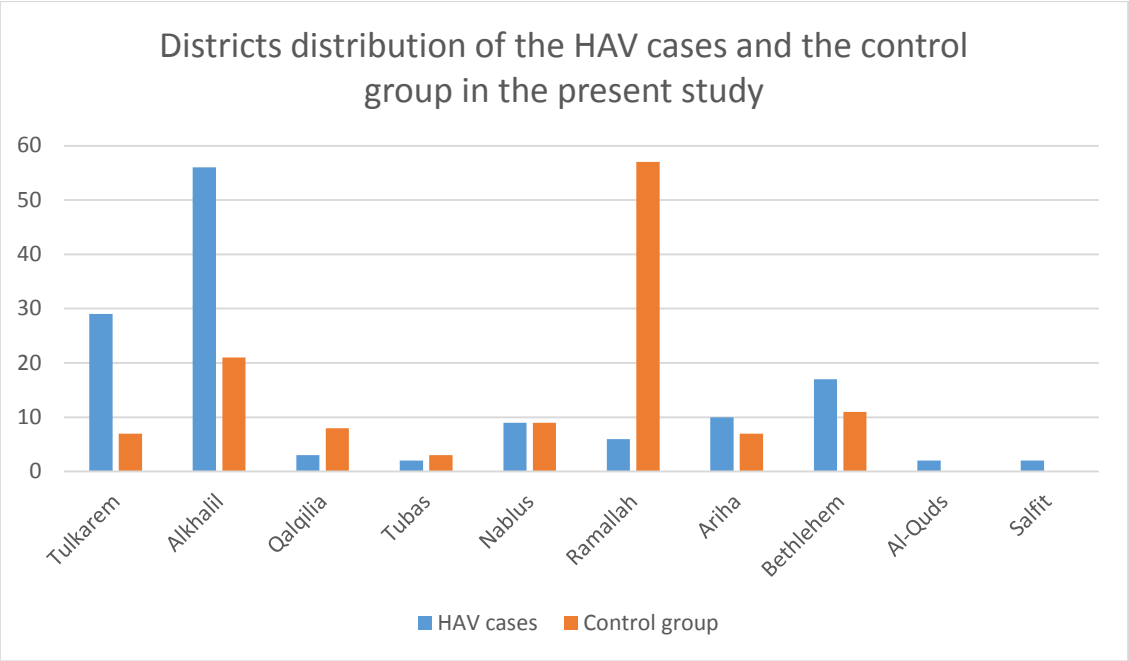

Fig.5: The number of cases and the control group from each district

3.2 RT-PCR, HAV genotypes and phylogenetic analysis results:

Of the 110 available serum samples of the HAV IgM cases, 68/110 (62%) yeild positive results for HAV RNA by RT-PCR (figure 6). Out of the 68 positive RT-PCR, only 18 samples were sequenced. All HAV strains were identified as subgenotype IB.

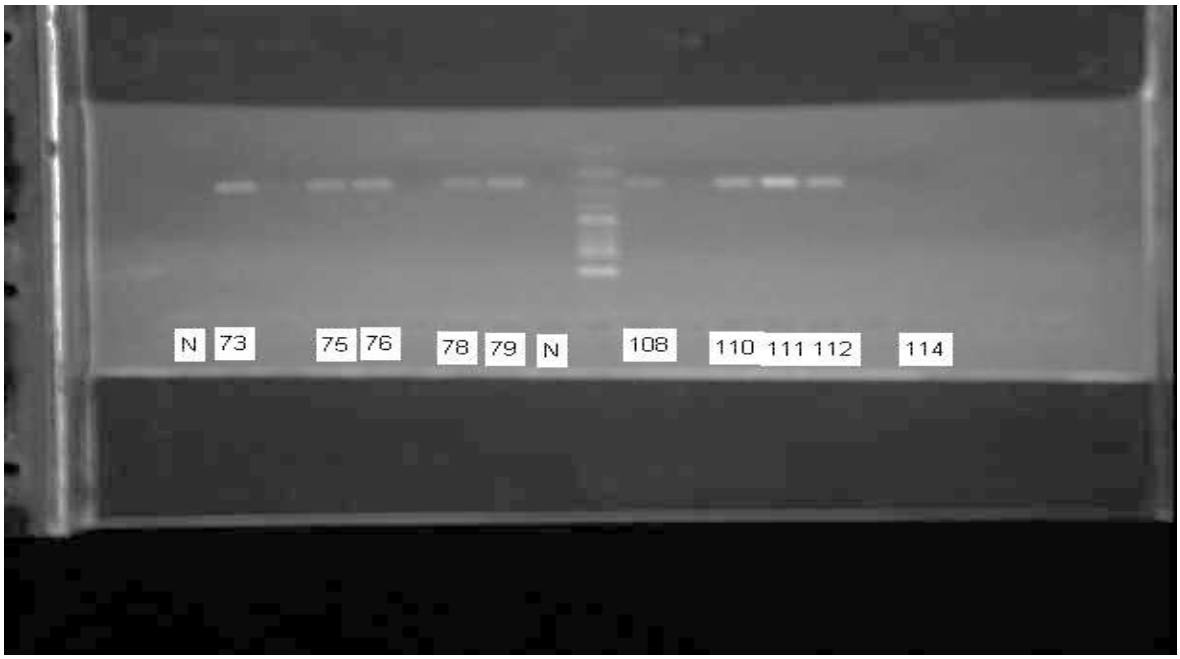

Fig.6. Agarose gel electrophoresis of selected PCR results

Phylogenetic analysis revealed that the sequences in the present study showed a closed homology with each other and form a separate cluster from other sequences retrieved from the gene bank Figure (7)

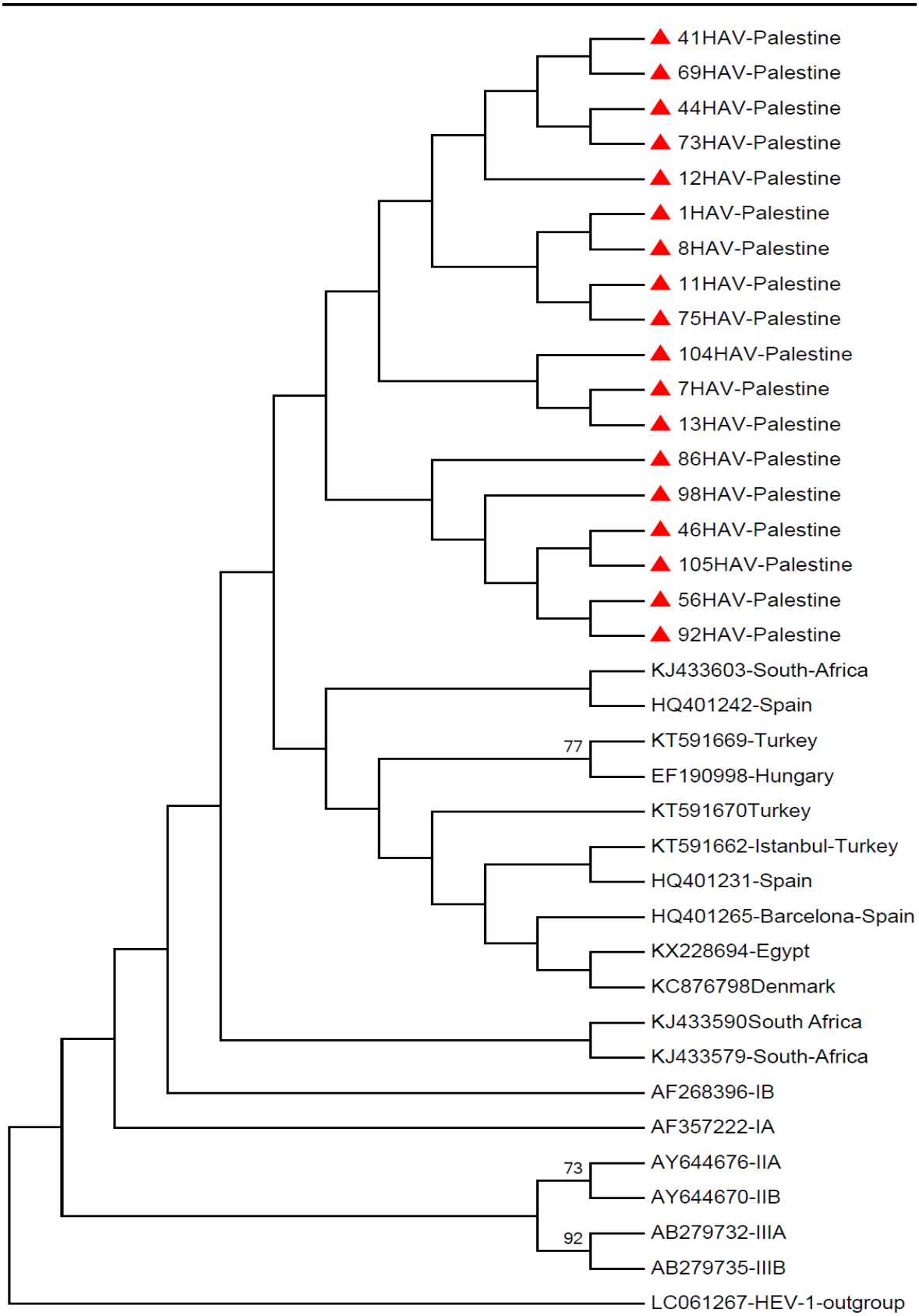

Figure.7: Molecular Phylogenetic analysis by Maximum Likelihood method.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura K. et al.,1993). The bootstrap consensus tree inferred from 1000 replicates (Felsenstein J, 1985). is taken to represent the evolutionary history of the taxa analyzed (Felsenstein J, 1985). Branches corresponding to partitions reproduced in less than 70% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above the branches (Felsenstein J, 1985). Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pair wise distances estimated using the Maximum Composite Likelihood (MCL) approach. The analysis involved 37 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 158 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura K. et al.,2013)

3.3Hepatitis A infections and associated risk factor in comparison with the control group:

The sex (male), occupation (student), educational level (Elementary-Basic) and Low income showed a significant difference for HAV infection in comparison to the control group (Table2).

Table 3.2: Risk factors associated with HAV infections

Sex	**Case**	**Control**	**Total**	**P value**
Male	82	63	145	**P=0.014^ S**
Female	54	73	127	
Occupation				**P=0.0001*S**
Student	**103**	36	139	
Housewife	**5**	28	33	
Worker	**9**	34	43	

Sex	Case	Control	Total	P value
Clerical worker	**1**	9	10	
None	**18**	28	46	
Level of education				**P=0.0001* S**
Elementary-Basic	99	49	148	
Middle	25	40	65	
University-High	12	47	59	
Income(NIS)				**P=0.0021^ S**
<= 1450	12	10	22	
1450-3000	90	68	158	
3000-5000	33	50	83	
5000	0	8	8	

***chi-square test; ^ Exact Fisher test; N: Number of subjects; NS: not significant; sig: significant.**

3.4 Comparison of sanitation practices, Personal Lifestyle and clinical history between HAV cases and the control group:

Drinking treated water, washing hand after defecation and before eating and eating unpeeled fruits were find to be associated significantly with HAV infection (P< 0.05). Furthermore, traveling abroad, visiting dental clinics and household contact of HBV and/or HCV infection were also find to be associated with HAV infection (P<0.05) as show in table3

Table 3.3: clinical and personal hygiene characteristics of272 subjects of the West Bank, Palestine.

Toilette	Case	Control	Total	P Value
In-house	132	136	268	**P=0.12^ NS**
In-yard	4	0	4	
Toilette link				**P=0.62^NS**

Toilette	Case	Control	Total	P Value
Septic tank	65	60	125	
Sewage network	71	76	147	
Treat drinking water				**P=0.03^ S**
Yes	1	8	8	
No	135	128	263	
Total	136	135	271	
Wash hands				**P=0.9^NS**
Yes	129	129	258	
No	4	5	9	
Wash hands after defecation				**P=0.0001^ S**
Every time	49	108	157	
Sometimes	87	28	115	
wash hands before lunch				**P=0.0001^S**
Every time	23	76	99	
Sometime	113	60	173	
Eat unpeeled fruits				**P=0.0022^S**
Yes	116	94	210	
No	20	42	62	
Travel abroad				**P=0.0003^S**
Yes	19	45	64	
No	116	91	207	
Dental procedure				**P=0.016^S**
Yes	31	50	81	
No	102	85	187	

Toilette	Case	Control	Total	P Value
Blood transfusion				**P=0.72^NS**
Yes	3	5	8	
No	133	131	264	
HBV/HCV Contacts				**P=0.007^NS**
Yes	18	5	23	
No	118	131	249	

^ Exact Fisher test; NS: not significant; sig: significant

Chapter Four:

Discussion and Conclusions:

4.1 Discussion:

Molecular markers have gained relevance as important tools for epidemiologic investigation, providing information on patterns of transmission and identifying the source in both sporadic and epidemic infections (Nainan et al., 2006).

The VP1-2A junction, is the most variable regions of the HAV genome, is generally chosen for phylogenetic analysis. In the present study, the VP1-2A junction of HAV was amplified and sequenced in order to determine the prevalent genotype in Palestine. The phylogenetic analysis with sequences derived from 18 RNA positive isolates revealed that all Palestinian HAV isolates were clustered on genotype IB

In many parts of the world, the anti-HAV seroprevalence rate is decreasing. Several factors are contributing to the declining infection rate, including increasing socioeconomic status, increasing access to clean water, and (in a few parts of the world) the availability of a hepatitis A vaccine that was developed in the 1990s. In some cases, this reduced force of

infection has significantly increased the average age at infection. This delay in viral exposure has created a large population of susceptible adolescents and adults at risk of disease and led to outbreaks of hepatitis A. Because the severity of infection increases with age, it may be appropriate for Hepatitis A Virus populations with a high proportion of susceptible adults to consider implementing vaccination programs targeted to certain populations often children. (WHO,2010)

Hepatitis A is one of the world's most common viral infections. Although most patients recover within two months, most cases of hepatitis A can be transmitted by fecal– oral contamination. It has been suggested that this association may largely reflect general living conditions and poor sanitation and hygiene, the decrease in number of patients suffering acute hepatitis was certainly in relation with the improvement of the socio-economic level and the hygiene conditions also Hepatitis A may be acquired from fecaly contaminated food or water and from wastewater-contaminated drills or water supplies (Hollinger, 1996). In our study, the age-distribution showed that HAV infection increased in age ≤10 .Children under the age of 10 years are particularly effective transmitters of hepatitis A infection and are usually asymptomatic. High concentrations of virons are shed in the stools of patients during 3 to 10 days prior to the onset of illness till one to two weeks after the onset of jaundice. Fecal excretion of HAV persists longer in children and in immunocompromised persons and may persist up to 4 - 5 months after infection than in otherwise healthy adults (Hollinger, 1996). Thus, young children should be the primary focus of vaccination. Also, the risk factors for HAV infection are higher in traveler, most travelers have to go through Jordan. It is containing people from all countries and different nationalities contained the largest number of refugees because of the political situation. so maybe persons who travel to countries are at substantial risk for acquiring hepatitis A, the sub-genotype IB detected in Jordan (Taylor,1997).

In these study HAV infection was transmitted by dental procedure, little risk exists for transmission of HAV and HEV from occupational exposure of dental hygienists to patients/clients infected with these viruses (Withers AJ, 1980).

Important source of infection is eaten raw or inadequately cooked food. Cooked food may Become recontaminated after cooking during inappropriate handling in our study unpeeled fruit may have been a source of HAV infection.

Finally, these findings demonstrate that hepatitis A represents a public health problem, requiring the investigation of the etiology and rapid action measures to control the infection.

4.2 Conclusion and Recommendation

In conclusion, genotype IB was found to be the prevalent genotype in Palestine. Despite the low diversity of HAV sequences, application of sequence analysis methodology is also important in countries lacking HAV genotype diversity, such as Palestine, to identify HAV outbreaks and to provide preventive healthcare measures for those in need, also HAV remain an important cause of hepatitis outbreak and is a major public worldwide health problem especially in developing countries. It is mostly reported from poor sanitary and unhygienic surroundings, which emphasizes the need for improving the public health measures to prevent epidemics of hepatitis A. The changes in epidemiological pattern would increase the disease burden, may cause large community outbreaks and lead to increased healthcare cost. The emergence of new serotype is highly unlikely, although new variants can emerge if virus population is forced to severe immune selective pressure. Since hepatitis A exists as a single serotype and human is the only host, it is possible to eradicate by selective vaccination against individuals who are susceptible and sero-negative for HAV IgM.

There is some Limitation in my study, Limited genomic sequencing of cDNA amplified by PCR from viral RNA so we need nested PCR to improve sensitivity and specificity and Financial aid is limited.

This is the first study describing the HAV genotypes and strains circulating in Palestine. Recommendation of vaccination depends of the HAV endemicity special on children under 10 years also improvement hygiene, sanitation, living standards, drinking water quality and adoption of regulatory measures of food handling would go a long way to interrupt HAV transmission

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Appendices :

Appendix A

HEPATITIS QUESTIONNAIRE			
Patient Name		Date of onset of symptoms/sampling	...../...../.....
DOB	...../...../.....	Sex	Male Female
Marital status	Single Married divorce		
Address	City/town/camps	District	
Level of education,	Elementary school Middle school University		
Occupation	.....		
Income	< 14500; 1450-3000, 3000-5000 >5000NIS		
Clinical history			
Have you ever had surgical procedure?	Yes No		
If the answer is yes, What type of surgical procedure did you do	.....		
Do you receive blood transfusion	Yes No		
If the answer is yes, when and where	.....		
Have you ever had a dentist procedures?	Yes No		
Have you ever had jaundice?	Yes No		
Do you have HCV?	Yes No		
Do you have HBV?	Yes No		
Do you have a house hold contact with HBV and/or HCV	Yes No		
Have you ever been abroad?	Yes No		
If yes, where?	.....		
Personal hygiene:			
Toilette	in house in yard		
Type	flush, open pits, bucket, …….		
Linked to	septic tank sewage system		
Drinking water source	Well, piped into house, piped into yard, spring, ...		
If not pipe lined,	do you treat water before drinking? Yes No; How:		
How often do you wash hands after defecation	Every time some times		
How often do you wash hands before lunch	Every time some times		
Eat raw vegetables	Yes No		
Eat unpeeled fruits	Yes No		
Swimming practice	Yes No		
Eating out side home	Yes No		
If yes, where			
Do you raise domestic animals?	Yes No		
	Yes, mention…….		
	No, have animals in vicinity Yes No		
Lab test results			
HAV IgM	Positive Negative		
HBS Ag	Positive Negative		
RT-PCR/stool	Positive Negative		
HCV Ab	Positive Negative		

Appendix B:

State of Palestine
Ministry of Health - Nablus
General Directorate of Education in Health

دولة فلسطين
وزارة الصحة– نابلس
الإدارة العامة للتعليم الصحي

Ref.: .....
Date:.....

الرقم: ..عح/١٦٣١/٢٠١٥..
التاريخ: ..٢٠١٥/٨/٢٩..

الأخ مدير عام الادارة العامة للرعاية الأولية المحترم،،،

تحية واحتراما،،،

الموضوع: تسهيل مهمة طلاب

تماشياً مع سياسة وزارة الصحة المتعلقة بتعزيز التعاون مع الجامعات والمؤسسات الأكاديمية بإتاحة فرص التدريب أمام الطلبة والخريجين والباحثين في المؤسسات الوطنية وإسهاماً في تنمية قدراتهم.

يرجى تسهيل مهمة **الطالبة حياة سميح قراقع – ماجستير علوم طبية مخبرية– جامعة القدس**، في بحث الماجستير بعنوان:" Seroprevalance and Molecular Characterization of Human Hepatitis A Virus (HAV) in Serum Samples of Palestinian Patients with Clinical Symptoms of Viral Hepatitis" من خلال السماح للطالبة بالحصول على معلومات من خلال استبانة، وجمع عينات من بقايا عينات المختبرات، وذلك في مراكز الرعاية الصحية الأولية في الوزارة في الضفة الغربية، مع العلم أنه سيتم الالتزام بمعايير البحث العلمي وسرية المعلومات.

- على ان يتم تزويدنا بنسخة من نتائج البحث.

مع الاحترام،،،

د. أمل ابو عوض
ق. أ. مدير عام التعليم الصحي

نسخة: د. رسمي الحلو المحترم/ جامعة القدس

P.O .Box: 14
TelFax: 09-2333901

ص.ب. 14
تلفاكس: 09-2333901

Appendix C:

بسم الله الرحمن الرحيم

عزيزي المريض.....

ارجو التكرم بالاجابة على اسئلة الاستبيان الذي تم اعداده لجمع المعلومات اللازمه لدراسة نسبة وجود التهاب الكبد الفيروسي أ بين سكان الضفه الغربيه.

يتم هذا البحث بالتنسيق مع وزارة الصحة الفلسطينية وجامعة القدس كمتطلب تخرج لانهاء درجة الماجستير في العلوم الطبية المخبرية مسار احياء دقيقه ومناعه.

ان تعاونكم وموافقتكم على المشاركة والاجابة على أسئلة الاستبيان سيكون له أهميه بالغه لانجاز هذا البحث علما بان المعلومات الخاصه بكم وبنتائج البحث ستكون في سرية تامه.

شاكرين تعاونكم

الباحثه : حياه سميح قراقع

.....

.....

بسم الله الرحمن الرحيم

اقرار شهادة

اسم المريض.....

التاريخ : / /

اشهد بان المذكور اعلاه قد اختار الاجابه على اسئلة الاستبيان بمحض ارادته بعد ان علم بان المعلومات ونتائج البحث ستكون سرية.

اسم وتوقيع الشاهد .....

التعرف على الوصف الجزيئي لفيروس التهاب الكبد الفيروسي البشري (أ) في عينات المصل من المرضى الفلسطينيين الذين يعانون من أعراض سريرية لالتهاب الكبد الفيروسي

إعداد : حياه سميح أحمد قراقع

المشرف : د. رسمي أبو حلو

الملخص

فيروس التهاب الكبدA هو عضو من عائلة *Picornaviridae* وهو فيروس وحيد والحمض النووي RNA له قياسه (27-32 نانوميتر).
الجسيمات المعدية لهذ الفيروس تتكون من البروتين وحمض نووي من نوع RNA ولها مناعة مدى الحياة بعد العدوى الطبيعية لأن لديه النمط المصلي المستضد واحد ولكن الاختلافات في تسلسل النيوكليوتيدات يمكن تصورها في منطقة الجينومVP1-2A يسمح تصنيف HAV إلى سبعة أنماط وراثية مختلفة (الأنماط الجينية الأول والثاني والثالث والسابع .(والتي تتسبب فيها هذه الأنماط الجينية المرتبطة بالعدوى البشرية، في حين أن الأنماط الجينية الرابعة والخامسة والسادسة تسبب الالتهابات في القرود، حيث ان النوع الجيني الاول مسؤول عن غالبية حالات التهاب الكبد الوبائي أ في جميع أنحاء العالم)

ينتقل الفيروس عبر الطعام أو الشراب الملوث ببراز شخص مصاب، ولذلك يمكن أن تحدث العدوى بعدة طرق: تناول طعام قام بتحضيره شخص مصاب بالتهاب الكبد (أ)دون أن يغسل يديه بعد الخروج من المرحاض، تناول فواكه أو خضار ملوثة بفضلات شخص مصاب، شرب مياه ملوثة بالفيروس، استخدام مرحاض مشترك مع شخص مصاب دون غسل اليدين جيدًا بعد الخروج، عدم غسل اليدين بعد تغيير حفاض طفل مصاب والعديد من الاسباب .

بعد ابتلاع المواد الملوثة بالفيروس ينتقل الفيروس عبر الطبقة الطلائية المبطن للبلعوم أو الأمعاءوتعبر من خلاله إلى الدم، وعبره تنتقل إلى الكبد حيث تستقر في خلايا الكبد وتتكاثر داخل هذه الخلايا، نتيجة لذلك تصبح هذه الخلايا عرضة لهجوم الخلايا المناعية.
يتم طرح كميات كبيرة من الفيروس عبر العصارة الصفراويةومنها إلى البرازوهكذا يصبح المصاب ناقلاً للمرض. تمتد فتر حضانة المرض ما بين 15 إلى 50 يومًا وتستمر الأعراض من أسبوع إلى أسبوعين.

لا يسبب التهاب الكبد الوبائي أ ضرراً دائماً للكبد وغالبا ما تمنح الإصابة بالتهاب الكبد الوبائي أ مناعة مدى الحياة، ينتج الجهاز المناعي الاجسام المضاده التي توفر الحصانة من المرض لاحقًا.

تم اجراء هذا البحث للكشف عن النمط الوراثي ولمعرفة نوع الجين المسؤول عن انتشار الفيروس في فلسطين ولمعرفة الوصف الجزيئي بوساطة علم الأمصال وهذا يفيدنا في امور كثيرة منها معرفة نوع التطعيم المناسب لاخذه في سن الطفوله بحيث ان اغلبية انتشار فيروس الكبد الوبائي أ تكون في سن مبكرة وأيضا لمعرفة مدى انتشاره في مناطق الضفه الفلسطينيهولاتمام هذه الدراسة تم جمع العينات من اشخاص مصابين بهذا الفيروس واشخاص ايضا سليمين للمقارنه بينهم من جميع انحاء مناطق الضفة الغربية في فلسطين ثم قمنا بتحليل العينات المصابه حيث قمنا بعمل فحص سيرولوجي ل 272 مريض كان منهم 136 ايجابي لفحصIgMحيث ان 75.74%منهم طلاب حيث

ان الفئات العمريه الاعلى كانت اقل من عشر سنوات ثم عملنا Reverse transcription PCR ثم DNA sequencing حيث نتج ان الجين المنتشر في فلسطين هو من نوع IB.