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Hepatitis E Virus Seroprevalence and Putative Risk Factors by a Cross- Sectional Study Among The General Population in West Bank, Palestine

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M.Sc. Thesis

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Thesis Approval

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Dedication

I dedicate this study to our Almighty God, who gave me the strength and knowledge to continue pursuing my goals despite many life obstacles.

I dedicate this study to my parents, who believed in me when I didn't believe in myself.

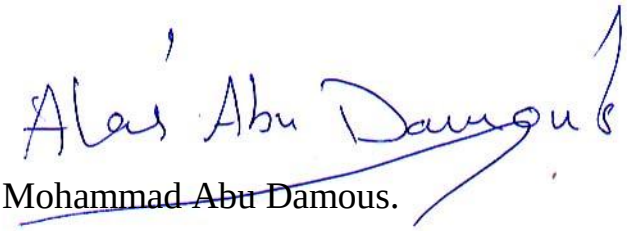
I dedicate this study to my sisters and brothers, who have always been the reason I wouldn't let myself give up.

I dedicate this study to my mentors, for always guiding me.

I dedicate this study to my friends, for their eternal love and support.

Declaration

I certify that this thesis submitted for the degree of Masters in Medical Laboratory Science 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: 

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Abstract:

Background: Hepatitis E virus (HEV) infection is a major public health concern in developing countries. HEV transmission occurs primarily by the oral-fecal route. This could progress to an acute self-limiting disease. Severe cases are more common among pregnant and immune-compromised patients, leading to a high mortality rate in these populations.

Objectives: This study aimed to evaluate whether the rate of seroprevalence of IgG anti-HEV and IgM anti-HEV are associated with sociodemographic variables, clinical history, personal hygiene ,and other related risk factors among the general population in West Bank ,Palestine .

Method: This seroepidemiological cross-sectional study was conducted from October 2016 to March 2017 include 432 participants from all governorates of West Bank, Palestine. Participants answered the questionnaire regarding the sociodemographic, clinical and personal hygiene characteristic. Blood samples were tested for IgG and IgM to HEV by an enzyme linked immunosorbent assay (ELISA). All serum samples positive for total anti-HEV were also assayed for IgM anti-HEV by ELISA.

Results: The overall seroprevalevce of anti-HEV was 3.7% (16/432). There was a significant association between anti-HEV seropositivity and levels of education. Anti-HEV reactivity among subjects with less than Tawjihi level (93.75% 15 out of 16) was higher than other group with high education level. Other associated factor with anti- HEV seropositivity, place of distract ($P< 0.0107$), the incidence rates of HEV seropositivity subjects were the highest in Ariha and Salfit (2/ 10= 0.20), but most cases of HEV seropositive subjects came from Al-Khalil populations 31.3% (5/16). Although participants aged ≥40 years had the highest prevalence, but there was no significant difference between the age groups. However, no significant relationship was observed between positive anti-HEV and other sociodemographic variables, travel, clinical

history (such as surgical procedures, dental procedures, and contact with hepatitis patients), and personal hygiene (such as type of sewage systems and history of animals contact). **Conclusion:** Although this study revealed low seroprevalence of HEV infection in the study population, the results showed that the HEV virus is circulating among the West Bank population of Palestine. This study points out the need for further studies to define the clinical and epidemiological importance of HEV infection and to identify additional risk factors involved in the epidemiology and pathogenesis of HEV infection.

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Abbreviations Used:

Hepatitis E Virus	**HEV**
Enzyme Linked Immunosorbent Assay	**ELISA**
Food and Drug Administration	**FDA**
Ministry of Health	**MOH**
Open reading frames	**ORFs**
Kilo base	**Kb**
Natural Killer cell	**NK**
Natural Killer- Like- T cell	**NKT**
World Health Organization	**WHO**
Nucleic acid test	**NAT**
Fulminate hepatic failure	**FHF**
Human chorionic gonadotropin	**HCG**
Human Leukocyte Antigen	**HLA**
Alanine aminotransferase	**ALT**
Aspartate aminotransferase	**AST**
Reverse transcriptase polymerase chain reaction	**rt- PCR**
Horseradish peroxides	**HRP**
Socioeconomic status	**SES**
Tetramethylbenzidine	**TMB**

Introduction:

Hepatitis E is a viral hepatitis caused by infection with hepatitis E virus (HEV). HEV is one of five human hepatitis viruses: A, B, C, D and E. HEV is a non-enveloped, positive sense, single stranded RNA (Fujiwara *et al*., 2014) as shown in figure1. It has been classified into the genus Hepevirus and has been reassigned into the Hepeviridae family (Aggarawal *et al*., 2012). Infection with hepatitis E virus was first documentedin 1955 in New Delhi, India during an outbreak (Aggarawal *et al*., 2011).

HEV is primarily transmitted through feces-contaminated water and eating uncooked or undercooked meat of HEV-infected animals. Person-to-person transmission occurs, but rarely via fecal-oral routes (Khuroo *et al*., 2015 and Vitral *et al*., 2014).

In developing countries, HEV is responsible for acute hepatitis outbreaks due to contamination of drinking water; however patients clear the virus rapidly. In developed countries, HEV is now considered as an emerging disease due to the increased number of autochthonous; chronic state HEV infection (Khuroo *et al*., 2015). Its severity varies from asymptomatic or self-limited, to fulminate. Mortality associated with hepatitis E infection is typically low, except when those infected are pregnant or immune-compromised (Teshale *et al*., 2010).

Therapy should be predominantly preventive. Practicing proper personal hygiene is essential, as well as avoiding drinking contaminated water and using good sanitation systems. There is currently no Food and Drug Administration (FDA)-approved vaccine for hepatitis E infection (Aggarawal *et al*., 2011).

Although there are no reports from the Palestinian Ministry of Health (MOH), indicating that there is a prevalence of hepatitis E in Palestine. This study was the first study done in West Bank, Palestine to determine the prevalence and risk factors of Hepatitis E infections among Palestinians in the West Bank.

Chapter One:

Literature Review

1.1. Virus Biology:

Hepatitis E is a viral hepatitis caused by the Hepatitis E virus (HEV), also known as enterically transmitted, non-A, non-B virus (Trinta *et al*., 2001 and Aggrawal *et al*., 2000). HEV is a member of the Genus Hepevirus and the Family Hepeviridae. As shown in figure 1 it is a positive-sense, single-stranded, non-enveloped RNA of approximately 7.5 kilobase (kb) (Verghese *et al*., and Yoon *et al*., 2014).

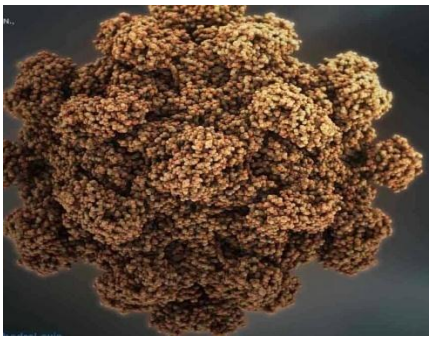

Figure 1: Hepatitis E Virus structure (Aggarwal *et al*., 2011).

1.2. Life cycle of HEV:

The knowledge on the HEV life cycle is limited, mainly because of the inefficiency of current cell culture models for HEV propagation (Schemerer *et al*., 2016).Cellular receptors and entry

modes of HEV into the host cell are not known, but it is known that HEV requires heparin sulfate proteoglycans to attach and infected target cells. The viral particles concentrated on the surface of hepatocytes are internalized after binding a specific receptor. The virus then sheds its coat to release genomic RNA that is translated into nonstructural proteins inside the cytoplasm. RNA-dependent RNA polymerases replicate the positive-sense genomic RNA into negative-sense transcripts; the latter then act as templates for the synthesis of a 2.2-kb subgenomic RNA, as well as full-length, positive sense transcripts. The positive-sense subgenomic RNA is translated into open reading frame (ORF) 2 and ORF3 proteins. The ORF2 protein packages the genomic RNA to assemble new virions, while the ORF3 protein may optimize the host cell environment for viral replication. The ORF3 protein is also associated with endomembranes, or plasma membranes, and may aid in viral egress (Khuroo *et al*., 2015) as shown in figure 2.

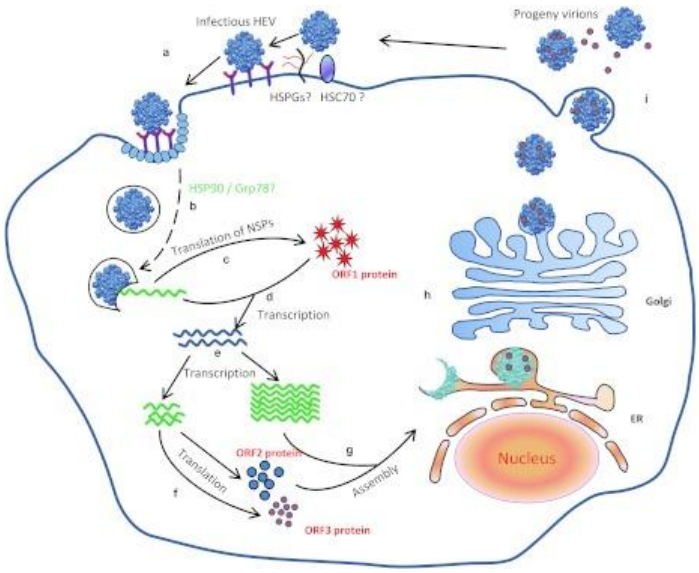

Figure 2 :Proposed life cycle of HEV.**Step a**: HEV attaches to the cell surface via HSPGs, HSC70 or other putative attachment receptor(s) and then enters the cell via unknown specific cellular receptor.**Step b**:. The virion then uncoats and releases the positive-sense genomic RNA into the cytoplasm of the cell. **Step c**: The positive-sense genomic viralRNA serves as the template to translate the ORF1 nonstructural polyprotein in the cytoplasm. **Step d**: The viral RdRp synthesizes an intermediate, replicative negative-sense RNA from the positive-sense genomic RNA that **step e:** serves as the template for the production of positive-sense, progeny viral genomes. **Step f**: The ORF2and ORF3 proteins are translated from the sub genomic, positive-stranded RNA, and **step g:** the ORF2 capsid protein packages the genomic viral RNA and assemblesnew virions. **Step h:** The nascent virions are transported to the cell membrane, and **step i:** the nascent virions are released from the infected cells. (Cao *et al.*, 2102).

1.3. Route of Transmission of Hepatitis E infection:

HEV can be transmitted horizontally by the oral-fecal route through fecal contamination of water supplies in regions with poor sanitation, food-borne transmission from ingestion of products derived from infected animals, and transfusion of infected blood products (although rare). Hepatitis E can also be transmitted vertically, through mother-fetus transmission. Finally, it is also known that there is also a possibility of zoonotic virus transmission (Banks *et al*., 2010, Colson *et al*., 2007 and Krain *et al*., 2014).

1.4. Typical Immune Responses to Hepatitis E Virus:

HEV is known as non-cytopathic. The liver injury during hepatitis E infections can be mediated by the host immune response. The direct cytopathic effect of the HEV has been difficult to study because the virus has not been cultured efficiently in-vitro. Both cellular and humoral mediated immunity play an important role in the pathogenesis of HEV infection. Cellular immune responses, both innate and adaptive immunity, are very important for viral clearance (Srivastava *et al*., 2007). Natural killer cells (NK), natural killer-like T cells (NKT), dendritic cells, and antigen-specific MHC class1-restricted $CD8^+$cells are involved in the lysing of virus-infected cells by producing IFN-γ (Szomolanyi-Tsuda *et a*l., 2002). NK and NKT cells are the major source of IFN-γ in the liver (Rehermann *et al*., 2003).

Patient suffering from HEV typically exhibit an increase in the total level of $CD4^+$, IFN-γ, and IL-1β. However, usually there is no increase in the levels of $CD8^+$, IL-2 and TNF-α. That suggests $CD4^+$ IFN γ secreting cells neither belong to T-helper cell type 1 nor to type 2 that are involved in the pathogenesis of HEV. Increased production of IFN-γ and the absence of $CD8^+$ cell response lead to nonspecific innate immunity, NK and NKT cells play a significant role in

pathogenesis of HEV (Srivastava *et al*., 2007). The humoral immunity by the aid of antibodies plays a crucial role in neutralizing the virus (Schofield *et al*., 2000). However, both IgM and IgG appear concomitantly with the development of the jaundice. Persistence of virus and exact role in protection against HEV reinfection are still unclear (Khuroo *et al*., 1993).

1.5. Epidemiology of Hepatitis E Virus:

According to World Health Organization (WHO), HEV causes around 20 million cases every year, with over 3 million of those being acute infection. It is also known that about 70000 deaths occur worldwide per year; related to hepatic failure. An estimated one-third of the world's population has been infected with HEV (WHO, 2014; Holla *et al*., 2013, and Rein *et al*., 2012). HEV is responsible for more than 50% of acute viral hepatitis in adults in endemic areas, and 1% in non-endemic areas. Due to these high numbers and the harmfulness of this infection, HEV represents an important public health concern in many developing countries, especially in those with high-risk populations (Arora *et al*., 1996 and Perez-Gracia *et al*., 2014).

There have been large numbers of epidemiological studies in different parts of the world, showing the wide variation in HEV seroprevalence patterns (see table 1). It has been found that anti-HEV antibodies are present in persons living in all geographical areas. Though the HEV seroprevalence rates are higher among developing countries, high prevalence rates are often reported from South Asia, Egypt in the Middle East, and the Far East, except Japan. Low rates are often found in developed countries, such as Europe and the Americas (Mushahwar *et al*., 2008, Aggarwal *et a*l., 2011 and Trinta *et al*., 2001).

Table 1: Prevalence of anti-HEV in the general populations

	Anti-HEV%	Reference
Asia		
Iran	10%	(Behzadifar *et al*., 2016)
Korea	5.9%	(Yoon *et a*l., 2014)
Bangladesh	22.5%	(Labrique *et al*., 2009)
Europe		
Franca	16.6%	(Mansuy *et al*., 2008)
Spain	1.1%	(Fogeda *et al*., 2012)
Italy	3.9%	(Scotto *et al*., 2014)
Africa		
South Africa	15.3%	(Tucker *et al*., 1996)
Nigeria	43%	(Ola *et al*., 2012)
Americans		
USA	21%	(Kuniholm *et al*., 2009)
Canada	3%	(Minuk *et al*. , 2007)

These differences between studies could be related to ecological, environmental, and/or cultural variations, as well as differences in the hygiene condition, education levels, access of safe water sources, and sanitary sewage systems. Other factors associated with anti-HEV seropositivity are animal contact, occupational activity, and method of washing fruit and vegetables.

1.6. General Epidemiology:

HEV has two distinct epidemiological profiles as quite different conditions:

1- As large outbreaks and epidemics cases of hepatitis E in areas of endemicity, infected with genotype 1 in Asia and Africa, genotype 2 in Mexico and Africa, resulting in high morbidity among pregnant women and young children (Traore *et al*., 2012 and Bayhan *et al*., 2016).

2- As isolated clinical cases, known as sporadic cases where no epidemic has been reported in industrialized areas infected with genotype 3 (Wang *et al*., 1999).

1.7. Seroprevalence of HEV in Blood Donors:

Providing safe blood is a major concern of any blood bank in the world. It has been reported in many countries that blood donors are potentially able to cause transfusion-associated hepatitis E (Boxall *et al*., 2006; Tamura *et al*., 2007, and Matsubayashi *et al*., 2008). Its true frequency, however, is underestimated because testing of blood donors is infrequent and due to the sometimes asymptomatic nature of the infection. Asymptomatic HEV infection makes nucleic acid tests (NAT) is a molecular technique used to detect a particular pathogen (virus or bacterium) in a specimen of blood or other tissue or body fluid. It does so by detecting and amplifying the RNA or DNA of the pathogen which makes extra copies of its nucleic acids to detect and prevent transfusion of contaminated blood donations. In Japan, HEV infection by blood transfusion has been reported in Hokkaido, which is regarded as a region with increasing danger of HEV infection. Therefore, and unfortunately, it is the only place in the world where donor blood is screened for HEV RNA (Vollmer *et al*., 2016).

1.8. Complicated Infection, Immune Response and Outcomes:

1.8.1. HEV Infection in Pregnancy:

HEV infection is usually associated with mild and self-limited diseases that can lead to fulminant hepatic failure (FHF). This severe form of disease is particularly common among pregnant women with HEV infection with a mortality rate varying from 20-25% during the second and third trimester of pregnancy. It has been reported to be associated with the mortality rate of 80%

in HEV-induced fulminant hepatic failure cases in this trimester. Moreover, HEV in pregnancy is associated with spontaneous abortion, still births, low birth weight, and preterm delivery (Mamum *et al*., 2009, Sepanlou *et al*., 2010 and Begum *et al*., 2010).

HEV has both a high incidence and severe course in pregnant women in some regions of HEV endemic areas, such as India (Patra *et al*., 2007). In other HEV endemic areas, such as Egypt, it has been shown to have a benign course with little or no morbidity. These differences could be the result of early childhood HEV exposures, producing immunity. These differences can also be explained by stating that the predominant HEV genotype in Egypt could be less virulent than those in Asia (Stoszek *et al*., 2006).

The high mortality rate in pregnancy has been thought to be secondary to the associated hormonal and steroid hormone, estrogen, progesterone, and human chorionic gonadotropin (HCG), increase during pregnancy and consequent immunological changes. These immunological changes include down regulation of p 65 component of NK with a predominant Th2 bias in the T cell response along with host susceptibility factors mediated by Human Leukocyte Antigen (HLA) expression. These steroid hormones may promote viral replication. These hormones also have a direct inhibition on hepatic cells, which may predispose to hepatic failure when exposed to infectious agents. Jilani *et al*. found that HEV infected pregnant women with FHF have lower CD4 counts and higher CD8 counts. They also observed that the level of steroid hormones were significantly higher than HEV-negative pregnant women.

Moreover, pregnancy is characterized by a state of maternal immune tolerance toward the fetus. T cell activity is reduced, leading to a decrease of cytokines production. Th2 responses increase and Th1 and immunological changes in the placenta down regulate antigen presentation. Finally,

recent studies showed higher viral load in HEV-infected pregnant women than in non-pregnant women (Navaneethan *et al*., 2008).

Some studies showed the percentage of seroprevalence of HEV infection among pregnant women: in Ghana 28.66% (Adjei *et al*., 2009), Goban 14.1% (Caron *et al*., 2008), Egypt 84.3% (Stoszek *et al*., 2006), India 60% (Oncu *et al*., 2006), 5.7% in rural pregnant women in Durango in Mexico (Alvarado-Espuivel *et al*., 2014) and 7.36% in Gorgon, North East of Caspian Sea (Tabarrae *et a*l. 2011).

1.8.2. Chronic HEV Infection:

Hepatitis E infection is an emerging clinical threat in developed countries, especially among the immunocompromised and individuals with underlying chronic liver disease, exhibiting the zoonotic pathway. To date, there are no known cases of chronic Hepatitis E in developing areas. However, most cases of Hepatitis E with progression to chronic hepatitis and chronic liver illness are being reported among HEV genotype 3 cases acquired in the developed areas. These chronic cases are almost exclusively among those who are on immunosuppressive treatment for solid organ transplant (Hoofnagle *et al*., 2012 and Kamar *et al*., 2014). Autochthonous HEV infection has an important cause of hepatitis among solid organ transplant recipients' progression to chronic HEV infection, defined as HEV viremia persisting for more than six month (Kamar *et al*., 2011).

Globally, chronic HEV infection has emerged as an important cause of morbidity among solid organ transplant recipients. Many studies in Germany, France, and the Netherland showed that 6-8 % of all unknown causes of liver dysfunction among recipient patients may be due to HEV infection, with more than 60% of these progressing to chronic liver hepatitis (Kamer *et al*., 2008, Haagsma *et al*., 2008, Pas *et al*., 2012; and Moal *et al*., 2013). This type of infection was also

associated with an increased risk for graft rejection, particularly among kidney transplant recipients (Sue *et al*., 2016).

Immune dysfunction was observed in cirrhotic patients, who presented decreased innate immune system activity with a reduction in NK cell activity (Chuang *et al*., 1991). Innate immunity also suppressed in advanced stages of liver fibrosis (Jeong *et al*., 2011). Moreover, HEV on its own can contribute to a down regulation of immune activity. Proteins encoded by HEV ORF 3 gene might reduce the host inflammatory response to create viral replication (Chandra *et al*., 2008). Secretion of immunosuppressive α1- microglobulin was increased in HEV ORF 3-protein, expressing cells potentially resulting in a protection of virus infected cells (Surjit *et al*., 2006).

Increasing the risk of acute hepatitis E that progresses the chronicity has relation with the importance of immune response against infection that protects the host cell all patients were immunocompromised and progressed to chronic hepatitis have significantly CD3 and CD4 lower counts. This finding highlights the importance of T-cell mediated immunity for pathogen clearance.

1.9. Diagnosis of Hepatitis E Infection:

The incubation period after exposure to the HEV ranges from 15-60 days, with a mean of 40 days. Immediately prior to the onset of clinical symptoms, HEV can be detected in the bloodstream for 1-2 weeks, and is shed in stool at 3-4 weeks before the elevation of aminotransferases enzymes. At the onset of clinical symptoms, HEV virus is lost from the blood stream, but continues to be shed in stool (Kamar *et al*., 2014).

HEV infection can be diagnosed by testing serum aminotransferases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), bilirubinemia, variable degree of rise in serum

billirubin (mostly conjugated); and a mild increase in serum alkaline phosphates. These are sensitive, but not specific indicators for liver injury. Specific diagnosis of Hepatitis E is based on serological assays for HEV antibodies. Anti-HEV IgM and IgG titers continue to increase in the asymptomatic phase (Figure3). The anti-HEV IgM titer peaks the symptomatic phase and declines after to baseline values with 3-6 months of symptomatic illness. The anti-HEV IgG titers remain detectable for some years. In endemic areas, detection of IgM suggests acute infection, whereas IgG indicates past infection. In non-endemic areas, IgG has also been used for the diagnosis of acute or recent infection. No diagnostic assays for HEV have yet been approved by the FDA (Aggarwal *et a*l., 2011).

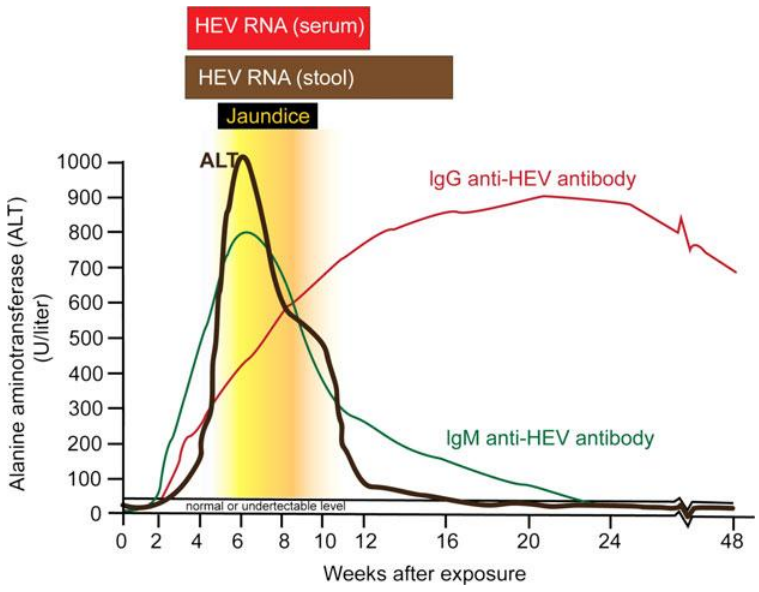

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Figure 3: Clinical, biochemical and serological profile of HEV infection (Khuroo *et al*., 2015).

Cases of Hepatitis E infection are not clinically distinguishable from other forms of acute viral hepatitis. Diagnosis of Hepatitis E virus infection is therefore based on the detection of specific

antibodies, virus in the blood stream. Enzyme linked immune-sorbent assay and western blot are used to detect anti-HEV IgM and IgG in serum. There are other assays: such as reverse transcriptase polymerase chain reaction (rt-PCR), to detect the HEV RNA in serum or stool, and immune-flouoresent antibody blocking assay to detect antibody to HEV antigen in the serum, and liver biopsies. Because of viremia and viral shedding, these assays may lack sensitivities.

1.10. Technical Issues Would Influence Anti-HEV Testing:

Currently commercially available serological assays are designed to detect anti-HEV Abs in human serum or plasma, based on their recombinant HEV proteins or synthetic peptides from ORF 2 and/or ORF 3 of HEV genotype 1 and 2. There may be a lower sensitivity for detection of infections with the genotype 3 in developed countries. Various reports indicate that commercial assays sometimes fail to detect specific anti-HEV genotype 3, and, thus, the numbers of autochthonous HEV infection in developed countries have been underestimated (Obriadina *et al*., 2002).

Most of these assays use recombinant polypeptides derived from the C and N terminal of ORF 2, or the C terminal of ORF 3. N and C terminal polypeptides from ORF 2 demonstrated greater abilities and were found to be more sensitive to detecting anti-HEV Abs than did those from ORF 3. This can be explained by the fact that anti-HEV against N and/ or C terminal polypeptides of ORF 2 appeared sooner than those against polypeptides ORF 3; thus, the N and C terminal polypeptides of ORF 2 may provide epitopes which are highly reactive with sera of acute HEV infection, while the C terminal polypeptides of ORF 3 may contain epitopes which are reactive with early convalescent phase sera, or from the later period of the acute phase (Ma *et al*., 2009). However, ORF 2-expressed proteins are believed to be more sensitive in detecting

anti-HEV IgM and IgG. In particular, the C terminal end of the ORf 2 region was found to contain a highly conserved conformational epitope and to be suitable for the specific and sensitive detection of anti-HEV Abs in ELISA assays (Chen *et al*., 2005).

1.11. Prevention HEV Infection:

The risk of infection and transmission can be reduced by ways such as good sanitation and through the availability of clean drinking water. The risk of infection can be reduced by establishing proper hygienic practices, such as hand washing with safe water, avoiding drinking water of unknown purity, avoiding eating raw or undercooked meat and fruits or vegetables that are not peeled or that are prepared by people living in or travelling in highly endemic area. The virus is resistant to heating at 56 °C for one hour. However, it is susceptible to boiling and frying for 5 minutes and to chlorination. Immunoglobulin is not effective in preventing HEV infections (Khuroo *et al*., 2015).

1.12. Objectives:

Main objective: The purpose of the study was to evaluate the prevalence of HEV infection and the risk factors among Palestinians in West Bank, Palestine.

Specific objective:

1- Determine the demographic and geographic distribution of HEV.

2 -Examine the presence of anti-HEV IgG and IgM in study using ELISA.

Chapter Two:

Materials and Methods:

This chapter outlines the research methodology used. It starts by explaining the research design and methods used, which include the study population and its eligibility criteria, sample size, the sampling technique used, recruitment process, the method of data collection used, data analysis methods, the validity and reliability of the research instruments, and ethical considerations.

2.1. Research Design and Study Population:

The study used a descriptive cross-sectional research design. The study population consisted of consenting individuals attending the primary health care centers of the Ministry of Health in West Bank, Palestine. These centers were selected for convenience because patients attending these centers usually reside within the same district where the center is located and visit the center for medical consultations, and maternal and child health care. Since there were no data on the prevalence of HEV in West Bank, Palestine, we calculated a sample size based on prevalence 50% (requiring the largest sample size) and with a precision of 5%. The minimum required sample size needed was 400 and 432 samples were tested. The tested samples were randomly selected to avoid any bias.

2.2. Eligibility Criteria

Individuals with positive results for hepatitis B and hepatitis C antibodies were excluded from the study to prevent an underestimation of the prevalence of anti-HEV seropositivity in the general population. Immunocompromized patients were also excluded.

2.3. Questionnaire:

A baseline questionnaire was applied to study participants to obtain behavioral characteristics, eating habits, contact with animals, blood transfusion, surgical and hospitalization history, and demographic, clinical, and socioeconomic status (SES) information which included level of education, income and occupation. 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. (See appendix A).

2.4. Specimen Collection, Transporting, and Preservation:

A total of 5 ml blood was collected from each subject into labeled sterile plain tube. Each blood sample was allowed to clot and the serum was harvested into a sterile tube. The serum was separated by centrifugation at 3500 rpm for 5 minutes. The serum samples were stored at -20 °C until use.

2.5. Serological Assays:

All serum samples were tested for total antibodies for HEV, IgM and IgG, by using the commercial Fortress HEV-Abs enzyme linked immunosorbent assay from MBS New S.R.L, Opera Company in Milano, Italy. All the serum samples that gave positive results from the previous assay were tested for HEV-IgM enzyme linked immunosorbent assay. Both assays utilize proprietary recombinant antigens, which is highly conserved among different HEV strains, to detect the presence of specific antibodies, including IgM and IgG against HEV.

2.5.1. BEM HEV-Ab ELISA Assay:

Principle of the assay:

1- HEV Total Abs ELISA:

This HEV-Ab ELISA kit uses microwell strips precoated with recombinant HEV antigens derived from conservative regions of ORF-2 of the native virus. In case of presence of HEV-Ab in the sample, the pre-coated antigens will be bound to the antibody during the first incubation step. After washing to remove unbound sample, second recombinant HEV antigen conjugated to Horseradish peroxides (HRP) is added into the wells. During the second incubation step, this antigen will bind to the second variable domain of the HEV Abs if they have been captured by HEV-antigen during the first incubation step. The unbound HRP-conjugate is removed during washing and chromogen solutions containing Tetramethylbenzidine (TMB) is added into the wells. In presence of the Ag-Ab-Ag (HRP), the colorless chromogens are hydrolyzed by the bound HRP-conjugate to a blue colored product. The amount of color intensity is proportional to the amount of Ab captured in the wells. Wells containing samples negative for HEV remain colorless.

2- HEV-IgM ELISA:

Principle of the Assay:In two-step incubation, solid phase ELISA assay, in which microwell strips are pre-coated with HEV-specific immuno-dominant synthetic antigens, directed to human immunoglobulin M proteins (anti-μ chain). During the first incubation step, any IgM-class antibodies will be captured in the wells. After washing out all ofthe other substances of the sample, IgG-class antibodies were blocked directly into the well by a neutralizing reagent. During the second incubation, the anti-hIgM antibody labeled with peroxidase (HRP) will specifically react only with HEV IgM antibodies. After washing to remove the unbound HRP-

conjugate, chromogen solutions were added into the wells. In presence of (HEV-Ag) - (HEV-IgM) - (anti-hIgM-HRP) immunocomplex, the colorless chromogens are hydrolyzed by the bound HRP-conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with0.3 M sulfuric acid.

ELISA Procedure for Total Anti-HEV and Anti-HEV IgM:

For total abs, 100 µL of Diluted specimens were added into each well expect the blank. 100 µL of positive control, negative control, and specimen were added into their respective wells except the blank. After 60 minutes of incubation at 37 °C, the wells were washed with diluted wash buffer (Dilute 1 to 25 with distilled water), 100 µL HRP-conjugate was added to all the wells, except blank, as second Abs and incubated for 30 minutes at 37 °C, washed with diluted wash buffer. 100 µL of chromogen/substrate to all the wells, including the blank and incubated for 15 minutes at 25 °C, blue color in the positive control and positive sample for HEV were produced by the enzymatic reaction. Then 100 µL of stop solution (0.3 M H_2SO_4) was added into each well, intensive yellow color was developed in the positive control and positive sample for HEV-Ab. Color development was measured at 450nm by using ELISA Reader (RT- 2100C Microplate Reader).while HEV- IgM assay procedure was the same, but 50 µL of neutralizing reagent was added in each well, except the blank and in the wells used for controls.

2.6. 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 test and fisher's exact test. The level of significance adopted for all tests was 5% ($p<0.05$).

2.7. Ethical consideration:

The study was granted ethical permission from the Ministry of Health, as shown in appendix B. Written informed consent was also obtained from all of the participants of this study, either by consenting for themselves or through parental consent, which is shown in appendix C.

Chapter Three:

Results:

Characteristics of study subjects and prevalence characteristics are described in this chapter. In addition, the findings regarding infectious risk factors for HEV infection are demonstrated.

3.1. Characteristics of the participants:

General participant's characteristics are summarized in Table 2. In total there were 432 subjects, 213 males and 219 females. The subjects were classified into five age groups: 0-9, 10-19, 20-29, 30-39, and over 40 years. 81 subjects were between 0 and 9 years old, while 77 subjects were between 10 and 19 years old, 84 subjects were between 20-29 years old, 84 subjects were between 30-39 years old, and 106 subjects were older than 40 years old. Among the participants, 9.26% lived in Jenin, 2.31% in Salfit, 25.23% in Al-Khalil, 4.63% in Qalqilia, 2.31% in Tubas, 15.51% in Nablus, 14.12% in Ramallah, 2.31% in Ariha, 12.5% in Al-Quds, and 7.41% in Bethlehem. Other results of the questionnaire are shown in Table 2.

3.2. HEV infection prevalence:

Overall, the prevalence of anti-HEV total antibodies was 3.7% (16/432). All the 16 subjects with positive results for anti-HEV total Abs were negative results for anti-HEV IgM Abs, so the prevalence of anti-HEV IgG Abs among a general population in West Bank, Palestine was 3.7%.

The sociodemographic status (SES), clinical and personal hygiene characteristics of the subject enrolled according to seropositivity for anti-HEV IgG are shown in Table 2, 3 and 4.

HEV seroprevalence was higher among males compared to females, but this was not statistically significant. Anti-HEV was distributed among all age groups. Although people aged ≥ 40 years had the highest prevalence, but there was no significant difference between the age groups.

However, with regard to place of district, anti- HEV IgG positivity was significantly associated with place of district (P= 0.0107), the incidence rates of HEV seropositivity subjects were the highest in Ariha and Salfit (2/ 10 = 0.20), but most cases of HEV seropositive subjects came from Al-Khalil populations 31.3% (5/16).

A similar pattern of a positive association of anti-HEV reactivity with education was found among study subjects (Table 2). Anti-HEV reactivity among subjects with less than Tawjihi level (93.75% 15 out of 16) was higher than other group with high education level. There was a significant statistical difference between them (P< 0.05).

In our study, no higher seroprevalence was found among people working in potentially risk professions such as health professional, then among those with less potentially risky jobs, such as clerical workers and students.

As shown in Table 3, there was no statistically significant association between anti- HEV IgG seropositivity and the presence of sewage system, source of water and toilette were taken into consideration (P> 0.05).

Of the clinical characteristics studies that were summarized in Table 4, seropositivity to HEV were not associated with blood transfusion, dental, surgical procedure, travel aboard and HBV/ HCV contacts.

Table 2: Sociodemographic characteristics of 432 subjects in the West Bank, Palestine, distributed according to seropositivity for anti-Hepatitis virus E (anti-HEV IgG) antibodies.

Characteristic	Anti-HEV positive N	Anti-HEV negative N	Total	P -Value
Gender				P= 0.1 NS
Male	11	202	213	
Female	5	214	219	
Total	16	416	432	
Age				P= 0.12 NS
0-9	4	77	81	
10-19	4	73	77	
20-29	1	83	84	
30-39	1	83	84	
≥40	6	100	106	
Total	16	416	432	
Place of District				P= 0.0178 Sig
Jenin	2	38	40	
Alkhalil	5	104	109	
Salfit	1	9	10	
Tulkarem	0	19	19	
Qalqilia	1	19	20	
Tubas	2	8	10	
Nablus	3	64	67	
Ramallah	0	61	61	
Ariha	2	8	10	
Alquds	0	54	54	
Bethlehem	0	32	32	
Total	16	416	432	
Education level				P= 0.04 Sig
<Tawjihi	15	288	303	
>Tawjihi	1	128	129	
Total	16	416	432	
Income				P=0.42 NS
<= 1450	3	49	52	
>1450	13	367	380	
Total	16	416	432	

Occupation				P= 0.48 NS
Student	3	96	99	
Housewife	4	77	81	
Worker	4	88	92	
Clerical worker	1	55	56	
HealthProfessional	0	27	27	
Teacher	0	25	25	
None	4	48	52	
Total	16	416	432	

N: Number of subjects; NS: not significant; sig: significant.

Table 3: Personal hygiene characteristics of 432 subjects in the West Bank, Palestine, distributed according to seropositivity for anti-Hepatitis virus E (anti-HEV IgG) antibodies.

Characteristic	**Anti-HEV positive N**	**Anti-HEV negative N**	**Total**	**P- Value**
Drinking water				P= 0.3137 NS
Well	3	35	38	
Bottle	0	10	10	
Pipe in house	13	367	380	
Total	16	412	428	
Toilette type				P= 0.58NS
Flush	10	289	299	
Open pit	6	127	133	
Total	16	416	432	
Toilette link				P= 1 NS
Septic tank	10	261	271	
Sewage network	6	155	161	
Total	16	416	432	
Swimming				P= 0.43 NS
Yes	4	153	157	
No	12	263	275	
Total	16	416	432	

Animal contact				P= 0.3 NS
Yes	10	202	212	
No	6	214	220	
Total	16	416	432	

N: Number of subjects; NS: not significant.

Table 4: Clinical characteristics of 432 subjects in the West Bank, Palestine, distributed according to seropositivity for anti-Hepatitis virus E (anti-HEV IgG) antibodies.

Characteristic	Anti-HEV positive N	Anti-HEV negative N	Total	P- Value
Surgery procedure				P=0.4 NS
Yes	7	131	138	
No	9	285	294	
Total	16	416	432	
Dental procedure				P= 0.7 NS
Yes	10	278	288	
No	6	138	144	
Total	16	416	432	
Blood transfusion				P= 0.61 NS
Yes	0	27	27	
No	16	389	405	
Total	16	416	432	
HBV/ HCV contact				P= 0.6 NS
Yes	1	27	28	
No	15	389	404	
Total	16	416	432	

Travel aboard			P= 0.60 NS
Yes	5	166	171
No	11	250	261
Total	16	416	432

N: Number of subjects; NS: not significant.

Chapter Four:

Discussion, Limitations and Conclusion:

4.1 Discussion:

Hepatitis E infection is a worldwide public health concern, which causes large out breaks of acute hepatitis in developing countries especially Asia, Middle East, and Africa and also sporadic cases of Hepatitis E in developed countries.

It was not possible to identify the etiological agent in some cases of viral hepatitis and those cases were classified as non-A, non-B, and non-C hepatitis infection. Therefore, it is possible some of those cases might have been HEV infection (Bortoliero *et al*., 2006).

For epidemiological purpose, subjects can be considered to approximate to the healthy general population. However, the age criteria for blood sampling and the elimination of samples found to be positive for hepatitis B and C infection could lead to an underestimation of the prevalence of anti-HEV seropositivity in the general population.

This study of HEV seroprevalence in West Bank, Palestine found that 3.7% of those sampled were positive, compared to, 2.81% the seroprevalence in Jewish populations (Karetnyi *et al*., 1995). This low prevalence is probably due to generally good hygienic conditions and controlled potable water supply as compared with other region.

The seroprevalence in other countries were 14.8% in Saudi Arabi (Abdelaal *et al*., 1998), 20% in United Arab Emirates (Kumar *et al*., 2001), 10.7% in Yemen (Bawazi *et al*., 2010) and 45.3% in

Egypt (Abdel-hady *et al*., 1989). These variations it can due to differences in the demographics of studied population, the size of the samples and the public health services situation. High rates of HEV infection were reported in Jordan, 30.9% (Obaidat *et al*., 2017), a country having a long border and exchange of population and livestock with Palestine. Jordan has been for many years the destination of immigrants coming from many countries, in the Gulf war, Jordan had a major role in it receiving soldiers from different parts of the world with ported high prevalence of HEV infection and with outbreaks. However, in Jordan the most frequent risk factors associated with increased anti-HEV seropositivity; eating camel's meat and being exposed to animals.

Several studies have been shown that seroprevalence of HEV in a population generally increases with age (Verhoef *et al*., 2012) which seems to be a risk factor for anti-HEV positivity (Kuniholm *et al*., 2009, Yoon *et al*., and Vitral., *et al*., 2014)). In this study, most cases of anti-HEV seropositivity distributed among aged groups ≥ 40 years old, but without significant association between aged groups, and it most likely reflects cumulative lifetime contact frequency to HEV. Also there some studies were shown no significant difference was found between different age groups (Bortoliero *et al*., 2006).

A high infection rate was found in high density district compared with standard density district. However, a large household constitute a risk factors, thus indicating a risk of person to person transmission. The key differences between inhabitants of densely-populated district are a generally lower economic status, with a lower level of education. As in this study, the incidence rates of HEV seropositivity subjects were the highest in Ariha and Salfit (2/ 10= 0.2), but most cases of HEV seropositive subjects came from Al-Khalil populations 31.3% (5/16) since 25% of the samples originates from this district. Contaminated water and possible zoonotic reservoirs

may play important role in the distribution of HEV seroprevalence among the general population.

Some studies have shown that the rate of HEV seropositivity is significantly higher in males than females (Xue-feng *et al*., 2008 and Takahashi *et al*., 2010), although some studies had shown higher rates in females (El-Tras *et al*., 2013), and other study shown no significant difference between males and females (Verhoef *et al*., and Mohebbi *et al*., 2012). In this study, HEV seroprevalence was higher among males compared to females, but this was not statistically significant.

In this study, other associated factors with anti-HEV seropositivity were levels of education. The results indicated that the rate of positive anti-HEV subjects increased with lower education levels (Oncu *et al*., 2006 and Mamani *et al*., 2015), it indicates the positive impact of education on sanitation and hygiene practice. However, previous studies have indicated that there was no difference in anti-HEV sreoprevalence according to educational level (Eick *et al*., 2010 and Ataei *et al*., 2009).

Sometimes, HEV infect human through direct contact with domestic or farm animals or contaminated water supplies. However, in this study there was no association between anti-HEV serpositivity and animals contact. The recognition of swine HEV infection in pigs in many countries of the world (Vitral *et al*., 2014) led to investigate a larger sample of pigs in Palestine, may act as reservoirs of HEV infection in Palestinian populations.

Some facts must be considered when studying the prevalence of HEV infection: we do not know the lifetime of antibodies for HEV and the test available for the detection of HEV antibodies seem to differ in their sensitivity and specificity. For these reasons it is difficult to determine the

true prevalence of HEV infection among any population, especially in regions of low endemicity.

4.2 Limitations:

A low prevalence of anti-HEV among the general population might affect the assessment of the risk factors associated with seropositivity for anti-HEV. The biggest challenges was faced , 25% of the samples came from Al-Khalil and this can cause bias in the interpretation of the results, so the prevalence rate was not used to explain the association between anti-HEV seropositivity and place of district. Instead of, the incidence rate was used for assessment to ensure that there is no bias in interpreting the results.

4.3 Conclusion:

Despite showing a low prevalence of HEV infection among general population in West Bank, Palestine, the results obtained in the present study demonstrate that HEV circulates in West Bank, Palestine. However, further studies are necessary to define the clinical and epidemiologic importance of the HEV infection in this area and to identify additional risk factors involved in the epidemiology and pathogenesis of HEV infection. The results of this study open the possibility of other routes of disease spreading different from that previously recognized ones such as fecal-oral contamination such as the possible role of animals in the transmission of HEV.

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Appendix A:

HEPATITIS QUESTIONNAIRE

Demographic Data			
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 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		
HEV Total Ab	Positive	Negative
HEV IgM	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: عحت/١٦٣٤/١٥ ت
التاريخ: ٢٠١٩/٨/٢٥ ت

الأخ مدير عام الادارة العامة للرعاية الأولية المحترم،،،

تحية واحترام...

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 آلاء ابو دعموس – ماجستير علوم طبية مخبرية– جامعة القدس، في بحث الماجستير بعنوان:" Seroprevalance for Hepatitis E Infection among Palestinians in the West Bank, Palestine" من خلال السماح للطالبة بالحصول على معلومات من خلال استبانة، وجمع عينات من المرضى المترددين على مختبرات التابعة للوزارة وذلك بعد اخذ الموافقة منهم، 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...

دولة فلسطين وزارة الصحة
الإدارة العامة للتعليم الصحي

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

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

ص.ب. 14
تلفاكس: 09-2333901

Appendix C:

بسم الله الرحمن الرحيم

عزيزي المريض----------

ارجو التكرم بالاجابه على اسئلة الاستبيان الذي تم اعداده لجمع المعلومات اللازمة لدراسة نسبه وجود التهاب الكبد الفيروسي ه بين سكان الضفة الغربية.

يتم هذا البحث بالتنسيق مع وزاره الصحة الفلسطينية وجامعة القدس وذلك كمتطلب تخرج لإنهاء درجه الماجستير في العلوم الطبية المخبرية. مسار أحياء دقيقة ومناعة.

أن تعاونكم وموافقتكم على المشاركة و الاجابة على اسئلة الاستبيان سيكون له أهميه بالغة لانجاز هذا البحث وكذلك لارتقاء بعلم الاوبئة بفلسطين علما بأن المعلومات الخاصة بكم وبنتائج البحث ستكون في سرية تامة.

شاكرين تعاونكم.

الباحثة: آلاء محمد طالب ابو دعموس.

بسم الله الرحمن الرحيم

اقرار شهادة

اسم المريض---------- التاريخ \ \

أشهد بأن المذكور أعلاه قد اختار الاجابة على أسئلة الاستبيان بمحض ارادتها بعد ان علم بأن المعلومات ونتائج البحث ستكون سرية.

اسم وتوقيع الشاهد:----------

معدل انتشار التهاب الكبد الوبائي (ه) و عوامل المخاطرة المفترضة من خلال دراسة قطاعية عرضية بين عامة السكان في الضفة الغربية ، فلسطين .

اعداد : ألاء محمد طالب ابو دعموس.

المشرف : د. رسمي أبو حلو .

الملخص

عدوى فيروس التهاب الكبد الوبائي (هـ) هي مصدر قلق رئيسي للصحة العامة في البلدان النامية . يعتبر مرض التهاب الكبد الفيروسي (هـ) مرض وبائي يصيب الكبد و ينتج هذا الالتهاب عن طريق فيروس التهاب الكبد هـ ,ينتقل هذا الفيروس الي الانسان عن طريق الفم بواسطه المأكل و المشرب الملوثيين . ولأن الفيروس يخرج من المصاب عن طريق برازه فعاده تكون سبب العدوى المباشرة هي شرب المياة الملوثة بمياه الصرف الصحي . وهذا يمكن أن يتقدم إلى مرض حادة ذاتي الحد .هذا المرض عادة ما يكون معتدلا في عامة السكان .الحالات الشديدة هي أكثر شيوعا بين الحوامل والمرضى المناعين للخطر، مما يؤدي إلى ارتفاع معدل الوفيات في هؤلاء السكان .

تم أجراء هذه الدراسة لتحديد و تقييم معدل انتشار المصلي للأجسام المضاده التي تكونت في أجسام المصابين ضد هذا الفيروس في الضفة الغربية في فلسطين . بحيث أجريت هذه الدراسة على 432 شخص من كل مناطق الضفة الغربية في فلسطين , وتم توزيع استبيان على المشاركون , وكل مشارك أجاب على الأستبيان الموزع الذي يتضمن الديموغرافيا الأجتماعية , النظافة الشخصية و السريرية . عينات الأمصال التي تم جمعها من 432 شخص تم فحصها للكشف عن الأجسام المضادة لكلية التي تكونت ضد التهاب الكبد(هـ)هن طريق تهاب الاستخدام تقنية الأليزا . فكل العينات التي وجدت ايجابية لوجود الأجسام المضادة الكلية ضد فيروس الكبد الوبائي (هـ), أيضا فحص للكشف عن الأجسام المضاده الحاده التي تكونت ضد هذا الفيروس باستخدام تقنية الأليزا. فكان الأنتشار المصلي 3.7% , حيث كان هناك ارتباط كبير بين ايجابية تكون الأجسام

المضادة ضد هذا الفيروس مع مستوى التعليم , كانت التفاعلية بين الأجسام المضادة وهذا الفيروس خلال الأشخاص الايجابين دون مستوى التوجيهي أعلى من المجموعة التي لها مستوى تعليمي عالي . بالنسبة لمكان انتشار هذا المرض الفيروسي الوبائي (هـ) في مناطق الضفة الغربية , الإلتهاب الكبدي الوبائي (هـ) أعلى في أريحا وسلفيت (10/2= 0.20)، إلا أن معظم حالات الإصابة بالفيروس المصاب بفيروس(هـ) جاءت من الخليل 31.3٪ (16/5). على الرغم من أن المشاركين الذين تتراوح أعمارهم بين≤ 40عاما كان أعلى معدل انتشار، ولكن لم يكن هناك فرق كبير بين الفئات العمرية. ومع ذلك لا يوجد علاقة كبيرة بين الأشخاص الذين كانو لديهم أجسام مضادة ضد هذا الفيروس في أمصالهم و التغييرات الديموغرافية, الأجتماعية الأخرى مثل (الحالة الأجتماعية , السفر , و النظافة الشخصية) . بالرغم أن هذه الدراسة كشفت انتشار مصلي قليل لعدوى فيروس التهاب الكبد الوبائي (هـ)في الأشخاص المشاركين, الأ أن النتائج بينت أن هذا الفيروس منتشر بين سكان الضفة الغربية في فسطين .

هذه الدراسة تشير الي الحاجة لمزيد من الدراسات الاضافية لتحديد الأهمية السريرية و الوبائية لهذه العدوى للتعرف على العوامل أخرى اضافية تودي الى هذا المرض و لها علاقة في علم الأوبئة و طريقة تطور المرض لعدوى التهاب الكبد الفيروسي الوبائي(هـ) .