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RESEARCH ARTICLE

THE PREVALENCE OF THE HEPATITIS C VIRUS IN THE WESTERN REGION OF LIBYA

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ABSTRACT

Background*:* Hepatitis C virus (HCV) is a primary cause of chronic liver disease worldwide. It is associated with the development of liver cirrhosis and hepatocellular carcinoma. Several studies have shown that patients infected with HCV may show variance in their response to different antiviral therapies, which suggests that more information about HCV is very important to physicians to help them to improve their services to patients. The study aimed to survey the prevalence of HCV in western Libya. ***Material and Methods****:* A total of 216 blood samples were collected from patients referred to Zawia reference center laboratory, Surman hospital, and the Center of Blood Bank in Sabratha. Samples were detected as HCV-positive using ELISA test. ***Results***: out of 216 patients ware 121 ware males (56%) and 95 ware females (44%). Their age ranged from 13 to 76 years, and they were distributed into four age groups, (1. 0-20 years, 2. 21-40 years, 3. 41-60 years, and 4. 60-80 years), thirteen of them were in the first group (6%), 86 in the second group (39.8%), 93 in the third group (43.1%), and 24 in fourth group (11.1%). All blood samples were subjected to ELISA test, and all of them were HCV positive. ***Conclusion***: The HCV infections are mainly in the age range of 21 to 60 (about 82.9%), which are more subjected to risk factors since they are at the age of working. Furthermore, people older than 60 years represent only about 11.1%, which suggests that HCV infections were less common earlier. The data also showed that males and females are equally vulnerable to the infection.

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INTRODUCTION

Since identified as non-A non-B hepatitis, by Choo and coworkers in the late 1980s, HCV has been the focus of many studies (Choo *et al.,* 1989; Wasley and Alter, 2000). HCV is a member of the Hepacivirus of the flaviviridae family (Robertson, *et al.,* 1998). The virus is a positive-sense, enveloped, single strand RNA of a proximately 9.6 Kb that codes a polyprotein with a single open reading frame (ORF) of 3008-3033 amino acid (Spinsanti *et al.,* 2008; Tanaka *et al.,* 1995). It is one of five known hepatitis viruses: A, B, C, D, and E (Georg *et al.,* 2001). Hepatitis C is an infectious disease caused by the hepatitis C virus (HCV) that primarily affects the liver (Wasley and Alter, 2000). During the initial stages of infection people often have mild or no symptoms. Occasionally a fever, dark urine, abdominal pain, and yellow tinged skin occurs. The virus persists in the liver in about 75% to 85% of those who have been initially infected. Early periods of the chronic infection typically has no symptoms. After many years however, HCV often leads to liver disease and occasionally cirrhosis (Choo *et al.,* 1989). In some cases, those with cirrhosis will develop serious complications such as liver failure, liver cancer, or dilated blood vessels in the esophagus and stomach (Wasley and Alter, 2000). The prevalence of Hepatitis C virus (HCV) is approximately 3% worldwide, whereas in Libya, according to one study involved more than 2900 participants from different populations suggested a very huge variations according to the risk factor involved; while it was as low as 1.6 % among the general population, it was as high as 20.5% among renal dialysis patients (Daw *et al.,* 2002; Naderi *et al.,* 2014). Recently, a comprehensive study was carried out on HCV genotypes in Libya. The study showed that Hepatitis C virus genotype 4 was the predominant one, followed by HCV genotype 1 and then other less common genotypes (Elasifer *et al.,* 2010). However, further studies are needed to clarify the magnitude and impact of HCV infections in Libya. HCV is spread primarily by blood-to-blood contact associated with intravenous drug use, poorly sterilized medical equipment, needle stick injuries in healthcare, and blood transfusions (Choo *et al.,* 1989; WHO, 1999). Using blood screening, the risk of HCV infection by blood transfusion is less than one per two millions (Choo *et al.,* 1989). It may also be spread from an infected mother to her baby during birth (Choo *et al.,* 1989). It does not spread by superficial contact (McOmish *et al.,* 1993). HCV diagnosis is

by blood testing to look for either antibodies to the virus or the viral RNA. A periodic testing is recommended for all people who have an occupation associated with blood or at risk (Choo *et al.,* 1989; McOmish *et al.,* 1993). Progression to chronic disease occurs in the majority of HCV-infected persons, and infection with the virus is one of the main causes of liver failure and liver transportation (Choo *et al.,* 1989; McOmish *et al.,* 1993). There is no vaccine against hepatitis C (Mohsen, 2001; Shoukry, 2018). Many ways are suggested to reduce and prevent HCV invections such as testing donated blood and reducing people who use intravenous drugs and (Spinsanti *et al.,* 2008). Chronic infection can be cured about 95% of the time with antiviral medications such as sofosbuvir or simeprevir (Choo *et al.,* 1989; Spinsanti *et al.,* 2008). The earlier treatments such as Peginterferon and ribavirin had a low cure rate of less than 50% and greater side effects, whereas the newer treatments can be expensive (Thomson *et al.,* 2005; Spinsanti *et al.,* 2008). The aim of this study is to investigate the distribution of the hepatitis C virus among the age groups and genders in the western region of Libya.

MATERALS AND METHODS

Samples collection: 216 samples were collected from public health services in three cities in the western region in Libya. These public health services are the Reference Laboratory in Zawia, Surman general hospital and Central Blood Bank in Sobratha. Blood samples were collected by venipuncture. The samples were allowed to clot naturally and completely, and the serum/plasma was harvested as soon as possible to avoid hemolysis of the RBCs. The serum samples were insured to be clear and not contaminated by microorganisms. Any visible particles were removed by centrifugation at 3000 RPMs for 10 minutes at room temperature.

ELISA Procedure: All reagents were used at room temperature (18-26˚C). Concentrated Washing Buffers were diluted 30-fold with distilled water (20 ml concentrated Washing Buffer add to 580 ml of distill water). Any precipitants were dissolved by warm up the concentrated washing buffer. Each reagent was well mixed before adding it to the test wells. The microwells needed on the ELISA Working Sheet were marked with the appropriate information. Positive and Negative Controls were run in duplicate to ensure accuracy. 100 ul of positive or negative controls were added to the appropriate wells. 100 ul of sample diluent were added to the test wells, then 10 µl of test samples were transfer to the test wells. The wells were gently rocked for twenty second, and then covered. The blank wells were kept as is. To ensure better precision, the solutions were well mixed with pipette. The wells were incubated for 30 minutes at 37°C. Carefully remove the incubation mixture by empting the solution into a waste container. Each well was filled with diluted wash buffer and shake gently for 20-30 second. The wash solution was discarded completely and the plate was tapped on absorbent paper. This procedure was repeat 4 more times. 100 ul (or 2 drops) of HRP- anti-human IgG conjugates were added into each well, covered, and incubated at 37°C for 20 minutes. The plate was wash 5 times as described in step 5. 100 ul (or 2 drops) of TMB substrate were add into each well. Incubated at room temperature (18-26˚C) in a dark place for 10 minutes. The reaction was stop by adding 50 ul (1 drop) of stop buffer to each well and gently mixed for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely. The microplate reader wavelength was set at 450 nm and the absorbance of each well was measured against the blank well within 15 minutes after adding the Stop Solution. A filter of 620 -690 nm was used as a reference wavelength to optimize the assay result.

Statistical analysis: Statistical analysis of the results using ANOVA, ($P \leq 0.05$) showed that a non-significant difference was found with F. value of (1.413**) between the number of patients infected with HCV. non-significant difference with F value of (1.413**), between the number of infected cases with HCV over the four different age groups. No significant difference between the infection rate in the two genders with F value of 1.413**.

RESULTS

Hepatitis C virus distribution in the study population: The study sample showed a distribution of HCV patients in all ages from 13 to 76 years with a mean of 41.4 years. The Data was collected as mentioned earlies from three different cities, the city of Surman where the data were obtained from Surman General Hospital, the city of Sabratha where the data were obtained from the Central Blood Bank, and the city of Zawia where the data were obtained from the Reference Laboratory of Zawia. The data represents the recorded cases in three consecutive years 2015, 2016, 1nd 2017. The total number of infected cases is 216 where males represented 56.5% and females represented 43.5%.

The Place of recorded cases: The data showed that the lowest number of cases were recorded in Surman with a total of 14 infected cases (about 6.5 % of the total), 8 of them are males and 6 of them are females, which could be due to the fact that Surman is much smaller than Sabratha city and Zawia city. Sabratha was second with a total of 51 infected cases (about 24 % of the total), 28 of them are males and 23 are females. Zawia had the Highest number of cases with a total of 151 infected cases (about 70 % of the total), 85 of them are males and 66 of them are females. Since Zawia is much larger than the other two cities, it is logical that it recorded more cases. The other thing that is worth to be mentioned is that number of female infected cases are less than the males in all of the cities with a male and female ratio in Surman 57 % males and 43 % female, in Sabratha 55 % males and 45 % females, and in Zawia 56 % males and 44 % females. Figure 1 summarizes these results.

The year of recording the cases: The data obtained was recorded in the three consecutive years 2015, 2016, and 2017(as it is presented in figure 2). The number of infected cases recorded in 2015 was 72 cases, 49 of them were males and 23 females. In 2016, the number of infected cases was 87 cases, 35 of them were males and 52 females. In 2017 the total number of infected cases was 57 cases, 37 of them were males and 20 females. The highest percentage of cases was recorded in 2016 (40.3 % of the total), whereas the second is in 2015 (33.3 % of the total), and the year 2017 had the lowest (26.4 % of the total). The number of infected females recorded in 2016 was higher than the infected males (about 60% females to 40 % males). In the year 2015 the ratio was 68 % males to 32 % females, and in the year 2017, the ratio was 65% males to 35% females.

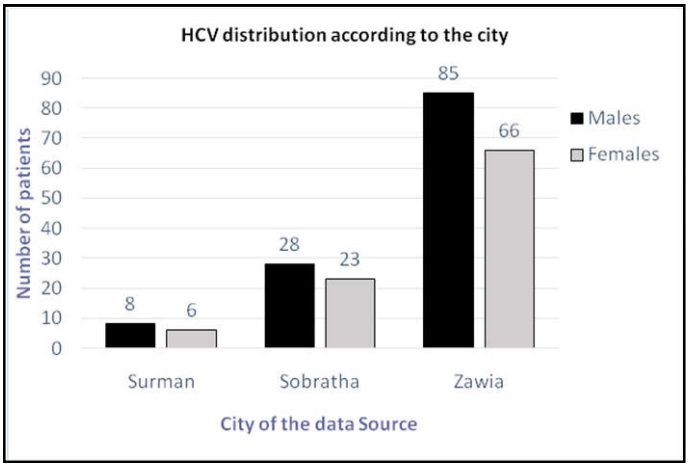

Figure 1. HCV distribution according to the public health services orientation. The data was expressed as the number of patients in each group. The first place is Surman general hospital with a total of 14 infected patients, 8 of them are males and 6 females. The second place is the Central Blood Bank in Sobratha with a total of 51 infected patients, 28 of them are males and 23 females. The third place is the Reference Laboratory in Zawia with a total of 151 infected patients, 85 of them are males and 66 females

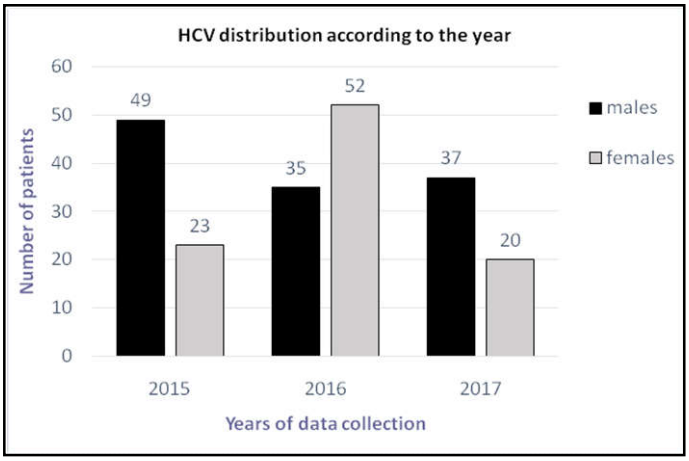

Figure 2. HCV distribution according to the year. The data was expressed as the number of patients in each year. The total number of infected patients in the first year (2015) was 72 persons, 49 of them are males and 23 females. The total number of infected patients in the second year (2016) was 78 persons, 35 of them are males and 52 females. The total number of infected patients in the third year (2017) was 57 persons, 37 of them are males and 20 females

The age group distribution: As it was mentioned earlier, the ages of infected cases were distributed from 13 to 76 years with a mean of 41.4 years. As it is shown in figure 3, the cases were divided according to their ages into 4 groups. The first group is from 0 to 20 years, the second group was from 21 to 40 years, the third group was from 41 to 60 years and the fourth group was 60 to 80 years. The number of infected cases in the first group (0-20 years) was 13 cases, 4 of them were males and 9 females. The number of infected cases in the second group (21-40 years) was 86 cases, 50 of them were males and 36 females. The number of infected cases in the third group (41-60 years) was 93 cases, 56 of them were males and 37 females. The number of infected cases in the fourth group (60-80 years) was 24 cases, 12 of them were males and 12 females. The first group (0-20 years) had the lowest number of cases representing about 6% with a female ratio of 69% and male ratio of 31%. The third group (41-60 years) had the highest number of cases representing about 43.1% of the total with a male to female ratio of 60.2% males and 39.8% females.

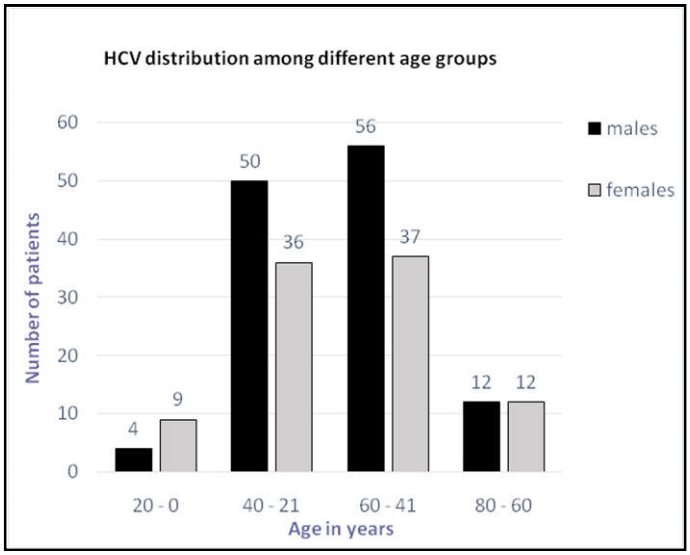

Figure 3. HCV distribution among different age groups. The data was expressed as the number of patients in each group. The first group is the young people aged under 20 years contains 13 patients, 4 of them are males and 9 females. The second is the age group 21 to 40 years contains 86 patients, 50 of them are males and 36 females. The third is the age group 41 to 60 years contains 93 patients, 56 of them are males and 37 females. The fourth is the age group older than 60 years contains 24 patients, 12 of them are males and 12 females

The second highest is the second group (21-40 years) representing about 39.8% of the total with a male to female ratio of 58.1% males and 41.9% females. The last group (60-80 years) had a total number of cases representing about 11.1% of the total with a male to female ratio of 50% males and 50% females.

DISCUSSION

HCV infection is one of the Flaviviridae infections with significant clinical problems throughout the world in humans, and it is responsible for the second most common cause of viral hepatitis (Leiveven and Pegasys, 2004). The virus genetically is very unstable and mutates rapidly. The virus can quickly become resistant to the specific anti-viral agents making treatment more difficult. In addition, with rapid mutations, an effective vaccine will also be a challenge. Data obtained from different parts of the world have focused on the increasing interest of HCV by mass screening as it is useful for answering the epidemiological questions and developing vaccines against HCV (Gómez-Escolar Viejo *et al.,* 2018). Furthermore, it has been shown to be beneficial to facilitate therapeutic decisions and strategies (McHutchison, 1999). The mass screening can also demonstrates the severity of the disease (Nausbaum *et al.,* 1995). In current studies, the frequency of HCV infections in Libyan population has been observed by testing provided serum samples by ELISA and proven to be positive for HCV (Trepo, 1994). In this study**,** comparing the three cities where the samples were collected reflected the size of the city. Zawia city, since it is the largest (its population is about 234000), recorded about 70% of the total HCV infections, and Surman the smallest (its population is about 36700), had only about 6.5%. Furthermore, the distance between Surman and Zawia is only about 15 km, and the distance between Sabratha and Zawia is about 24 km which means people can easily go from one place to another.

Comparing the three consecutive years that are represented in this study showed a slight increase in the year 2016 with a percentage of about 39.3%. The year 2015 had a percentage of 33.3% and the year 2017 had a percentage of 26.4%. The differences among these three years within the normal variation and we can conclude that the HCV virus spreads in a steady rhythm. The most commonly detected HCV infections in this study were age groups (41-60) with 43.1% and (21-40) with 39.8%, The total infected cases of these two groups represents about 82.9% of the whole infected cases. This result shows that these two groups are more likely to be infected than the other two groups since the risk factors for these two groups are greater because they are at the working age. This result agrees with that obtained by Alter and his group where they found that 76% of the infected cases in United States are older than 20 years (Alter *et al.,* 1999), even though in this study only 6.5% of the cases are in the age group 20 years or less which might be due to the cultural differences or since it is a silent disease some infected persons are not aware that they have been infected. Furthermore unlike other age groups, in this group more females are infected (69%) compared to males (31%). Other reports in Arabic countries revealed that the prevalence of HCV infection was mostly associated with the age group of (29-44). In Egypt Ray et. al., demonstrated the predominance of age group (29-44) (Ray *et al.,* 2000). Another study performed by Fakeeh and Zaki in Jeddah, in Saudia Arabia about the prevalence and common HCV among ethnic groups and they concluded that age group (29-44) is common (Fakeeh and Zaki, 1999). In Gazza Strip, Shemer-Avni *et al.* reported that HCV-G4 predominates (Shemer-Avni *et al.,* 1998). In Lebanon a study conducted by Rami *et al.* in Libanese thalassemics and the results indicated the predominance of age group (29-44) (Ramia *et al.,* 2002). Similar conclusion obtained by other authors in Syria, from hemodialysis patients (Abdulkarim *et al.,* 1998) and in Kuwait (Pasca *et al.,* 2001).

Libya is surrounded by six countries where HCV is endemic, including Egypt, which has the highest prevalence of HCV in the world. Close proximity to these countries could provide transmission arteries for HCV infection into and across Libya (Kalipeni and Zulu, 2012**).** Immigration and political instability are particularly important factors in HCV prevalence in Libya. Beside age, patient's gender is another demographic parameter that is linked to HCV prevalence and incidence in Libyan population. Previous study has shown that males are more susceptible to all genotypes of HCV than females and subsequently they showed higher HCV infection than females (Hana *et al.,* 2010). In another study conducted on a total 114,928 HCV infection cases during a ten-year period showed that HCV infection was higher among males than females aged between 25 and 39 years (Daw *et al.,* 2018). In agreement with previous studies, our results showed that higher incidence of HCV infection among males than females. The percentage of infected males was 56.5%, whereas the percentage of infected females was 43.5%. This could be attributed to that males are more exposed to different risk factors than females. Additionally, males are more sensitive to all genotypes of HCV than females.

Conclusion

We conclude from the above data that the HCV infections are mainly in the age range of 21 to 60 (about 82.9%), which are more subjected to risk factors since they are at the age of working. Furthermore, people older than 60 years represent only about 11.1%, which suggests that HCV infections were less common earlier. Unlike what was expected since kidney dialysis is more common in this age group. The data also showed that males and females are equally vulnerable to the infection even though infections in males are slightly higher than infections in females.

REFERENCES

Abdulkarim, A. S. Zein, N. N. Germer, J. J. Kolbert, C. P. Kabbani, L. and Krajnik, K. L. 1998. Hepatitis C virus genotypes and hepatitis G virus in hemodialysis patients from Syria: identification of two novel hepatitis V virus subtypes. *Am J Trop Med Hyg*., 50:571—6.

Alter M.J. 1999.The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. N *Engl J Med.* 341(8):556–62.

Choo, Q. L. Kuo, G. Weiner, A. J. Overby, L. R. Bradley, D. W. and Houghton, M. 1989. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science*, 244:359–364.

Colin C. 2001. Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature. *J. Viral. Hepat.,* 8: 87-95.

Contigiani M Nested PCR Enferm Infecc Microbiol Clin., 26(1):10-15.

Daw MA, Elkaber MA, Drah AM, Werfalli MM, Mihat AA, Siala IM. 2002. Prevalence of hepatitis C virus antibodies among different populations of relative and attributable risk. *Saudi Med J.,* 23 (11): 1356-1360.

EASL International Consensus Conference on hepatitis C. Consensus statement. *J. Hepatol.,* (1999). 31(suppl 1):S3– 8.

Elasifer HA, Agnnyia YM, Al-Alagi BA, Daw MA. 2010. Epidemiological manifestations of hepatitis C virus genotypes and its association with potential risk factors among Libyan patients. *Virol J.,* 7: 317-10.1186/1743-422X-7-317.

Fakeeh, M. and Zaki, A. M. 1999. Hepatitis C: prevalence and common geno-types among ethnic groups in Jeddah, Saudi Arabia. *Am J Trop*., 61:889—92.

Fakeeh, M. and Zaki, AM. 1999. Hepatitis C prevalence and common genotypes among ethnic groups in Jeddah, Saudi Arabia. *Am. J. Trop. Med. Hyg.,* 61:889-892.

Georg, M. Lauer, M. D. Bruce, D. and Walker, M. D. 2001. Hepatitis C Virus Infection N. E. J. M. 345:41-52.

Gómez-Escolar Viejo L, García Herola A, Sáez Lloret I, Sánchez Ruano F, Clemente Paulino I, Quílez Ivorra C, Almenta Saavedra I, Martínez Pérez D, Valverde de la Osa 2018. *J.Eur J Gastroenterol Hepatol.,* 1077-1081.

Hana A Elasifer, Yossif M Agnnyia, Basher A Al-Alagi and Mohamed A Daw, 2010. Epidemiological manifestations of hepatitis C virus genotypes and its association with potential risk factors among Libyan patients. *Virology Journal,* 7:317.

International Consensus Conference on Hepatitis C: Paris, 1999. consensus statement. *J. Hepatol.,* 30:956-961.

Kalipeni E, Zulu L. 2012. HIV and AIDS in Africa: a geographic analysis at multiple spatial scales. *Geo Journal,* 77:505–23

Lavanchy, D.. 1999. Global Surveillance and Control of Hepatitis C. *Journal of Viral Hepatitis,* 6:35-47.

Leiveven, J. and Pegasys, R. B.V. 2004. Improves Fibrosis in Responders, relapsers and Non responders with Advanced

Fibrosis. *55th Annual Meeting of the American Association for the Study of Liver Disease*. Boston, MA. USA.

Love, RA. Parge, HE. and Wickersham, JA. 1996. The crystal structure of hepatitis C virus NS3 proteinase reveals a trypsin-like fold and a structural zinc binding site. *Cell*, 87:331-342

McHutchison, JG. Poynard, T. Davis, GL. Esteban-Mur, R. Harvey, J. Ling, M. Cort, S. Fraud, JJ. Albrecht, J. and Dienstag, J. 1999. Evaluation of hepatic HCV RNA before and after treatment with interferon alfa 2b or combined with ribavirin in chronic hepatitis. *Hepatology*, 30:363A.

McOmish, F. Chan, SW. Dow, BC. Gillon, J. Frame, WD. and Crawford, RJ. 1993. Detection of three types of hepatitis C virus in blood donors: investigation of type-specific differences in serologic reactivity and rate of alanine amino transferase abnormality Transfusion, 33: 7-13.

Mohamed A. Daw, Lutfi A. Buktir Ali, Amina M. Daw, Nadia E. M. Sifennasr, Aghnyia A. Dau, Mohamed M. Agnan, Abdallah El-Bouzedi and In association with the Libyan Study Group of Hepatitis and HIV. 2018. The geographic variation and spatiotemporal distribution of hepatitis C virus infection in Libya: 2007–2016. BMC Infectious Diseases 18:594.

Mohsen, AH. 2001. Trent HCV study group. The epidemiology of hepatitis C in a UK health regional population of 5.12 million. Gut. 48:707–713.

Naderi, M., Gholipour, N., Zolfaghari, M. R., Moradi Binabaj, M., Yegane Moghadam, A., and Motalleb, G. 2014. Hepatitis C virus and vaccine development. *International journal of molecular and cellular medicine*, *3*(4), 207-15

National Institutes of Health Consensus Development Conference Panel statement: management of hepatitis C. Hepatology, 1997. 26: Suppl 1:2S-10S.

National Institutes of Health Consensus Development Conference Panel statement: management of hepatitis C. Hepatology, (1997). 26: Suppl 1:2S-10S.

Nausbaum, JB. Pol, S. Nalpas, B. Landras, P. Berthelot, P. and Brechot, C. 1995. Hepatitis C virus type 1b(II) infection in France and Italy. *Ann Intern Med.*;12:161–8.

Pasca, A. Al-*Mufti*, S. Chugh, T. and Said-Adi G. 2001. Genotypes of hepatitis C virus in Kuwait. *Med Princ Pract.,* 10:55—7.

Pawlotsky JM. 2002. Use and interpretation of virological tests for hepatitis C. Hepatology. 36 (Suppl 1): S65-73.

Pawlotsky, JM. 1998. What strategy should be used for diagnosis of hepatitis C virus infection in clinical laboratories?. *Hepatology*, 27: 1700-1702.

Pawlotsky, JM. Bouvier-Alias, M. Hezode, C. Darthuy, F. Remire, J. and Dhumeaux, D. 2000. Standardization of hepatitis C virus RNA quantification. *Hepatology,* 32:654 – 659.

Ramia, S. Koussa, S. Taher, A. Haraki ,S. Klayme, S. and Sarkis D. 2002. Hepatitis C virus genotypes and hepatitis G virus infection in Lebanese thalassemics. *Ann Trop Med Parasitol*., 96:197— 202.

Ray, S. C. Arthur, R. R. Carella, A. Bukh, J. and Thomas, D. L. 2000. Genetic epidemiology of hepatitis C virus throughout Egypt. *J. Infect Dis*. 182:698—707.

Robertson, B. Myers, G. Howard, C. Brettin, T. Bukh, J. Gaschen, B. Gojobori, T. Maertens, G. Mizokami, M. Nainan, O. Netesov, S. BCJG Nishioka, K. Shin,I.T. Simmonds, P. Smith, D. Stuyver, L. and Weiner, A. 1998. Classification, nomenclature, and database development for hepatitis C virus and related viruses: proposals for standardization. International Committee on Virus Taxonomy [news]. Archives of Virology 143:2493-2503.

Shemer-Avni, Y. Astal, Z. Kemper, O. el Najjar, K. J. Yaari, A. and Hanuka, N. 1998. Hepatitis C virus infection and genotypes in Southern Israel and the Gaza Strip. *J Med Virol*., 56:230-3.

Shoukry N. H. 2018. Hepatitis C Vaccines, Antibodies, and T Cells. *Frontiers in immunology*, *9*, 1480. doi:10.3389/ fimmu.2018.01480

Spinsanti, L. Farías, A. Díaz, A. Vázquez, A. Aguilar, J. and Tenorio, A. 2008.

Tanaka, T. Kato, N. Cho, M. J. and Shimotohno, K. 1995. A novel sequence found at the 3 terminus of hepatitis C virus genome. *Biochemical and Biophysical Research Communications*, 215:744-749.

Thomson, BJ. and Finch, RG. 2005. Hepatitis C virus infection. *Clin Microbiol Infect.,* 11:86–94.

Trepo, C. 1994. Seminar on hepatitis C; European Commission Public Health Unit.

Wasley, A. and Alter, M. J. 2000. Epidemiology of hepatitis C: geographic differences and temporal trends. Semin. *Liver Dis.,* 20:1–16.

WHO, 1999. Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. *J. Viral*. *Hepat.,* 6:35–47.

World Health Organization, 2000. Hepatitis C - Global Surveillance Update. *Weekly Epidemiological Record,* 75:17-28,

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