Genotypes of Hepatitis C Virus in United Arab Emirates: Their Relationship with Age, Gender and Nationality

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Abstract
Background: Hepatitis C virus (HCV) is a major global public health problem with estimate of 3% chronicity. The prevalence of HCV in Eastern Mediterranean Region was variable with a range of 1% to 14.9%. HCV infection was found to be endemic in Arab World as different studies confirmed such endemicity. Arabian Peninsula region HCV prevalence was reported as low (1-1.9%) in Oman, Saudi Arabia, Kuwait, and Bahrain, while it was moderate in Yemen (2.0-2.9%), high in UAE (3.0-3.9%), and very high in Qatar (>4%).
Aim: To study sero-molecular epidemiology of HCV in UAE genotypes among the patients who have HCV positive antibodies and differentiate them according to their nationality in United Arab Emirates.
Patients and methods: The study was conducted from June 2013 to December 2017 at the Medical as well as Infectious Diseases clinics at Khalifa Hospital Abu Dhabi, UAE. The patients with positive screening test for HCV antibodies referred from other clinics and peripheral health centers were included in the study. The study was designed to include patient's demographics, clinical information including the various risk factors for the transmission of HCV and laboratory data which included serum HCV RNA levels, HCV Genotypes and Liver function test (LFTs). A blood sample of each patient was collected and the confirmation of HCV was done by Western blot. The confirmed cases were further tested for HCV RNA levels by polymerase chain reaction and subsequently HCV-RNA positive patients were genotyped by selective hybridization of amplicons to HCV genotype-specific oligonucleotides.
Results: A total of 193 patients included in the study with a mean age of 44.23 years and 76.68% of them were male, with M/F ratio of 3/1. The highest frequency was in Egyptian (60.10%), followed by UAE (18.13%) and 21.76% in others. The predominant genotype was 4 (67.93%), type 1 form 16.85%, and type 3 form 15.22%. Gender with significant influence on HCV genotype (P=0.001). HCV type 1 was higher in female (40.48%) than in male (9.86%), while type 3 was higher in male

(17.61%) than in female (7.14%), also type 4 was more frequent in male (72.54%) than in female (52.28%). HCV genotype 4 was the predominant types in Egyptian (97.35%), while type 1 in UAE citizen (68.57%), and type 3 in others (63.89%). Age was not with significant influence on genotype frequency, however, gender, nationality, and disease severity was with significant association between HCV genotype and age, gender and nationality (X^2 =179.01; P=0.001). Female gender was with negative association with type 3 genotype and female with relative risk to infection with type 4 genotype11 times to infection with type 3 genotype. There is no observed Egyptian with type 3 genotype infection in our study cohort.

Conclusion: HCV more predominant in Egyptian than in UAE. However, Egyptian has a significant negative correlation [OR=0.085, P=0.006] with type 1, while UAE are with significant positive correlation with genotype 1 after excluding the effect of gender. Furthermore, Egyptian is 11 times susceptible for genotype4 than for genotype1, while the Emirati are 10 times susceptible for genotype1 than for genotype4. However, the genotype frequency distribution indicated that HCV infection in Emirati was with significant association with type 1 (OR=10.246; p=0.003), while OR was 0.085 in Egyptian and thus the hypothesis that presumed the increase in prevalence of HCV in Emirati was excluded. In addition, type 3 genotype was with 0% frequency in Egyptian, while it forms 63.89% as a cause of HCV infection in other nationality and thus this finding is strong evidence that exclude Egyptian a cause for increase of hepatitis C in Emirati.

Key words: Hepatitis, HCV, Genotype, Nationality, United Arab Emirates, Egyptian.

Introduction

Hepatitis C virus (HCV) is a major global public health problem with estimate of 3% chronicity [1]. The prevalence of HCV in Eastern Mediterranean Region was variable with a range of 1% to 2.5% in most countries, with a high prevalence reported in Egypt (>10%) [1]**,** and in Libya, Sudan and Yemen (2.5%-10%) [2]**.** Middle East and North Africa region appears to have the highest prevalence of HCV infection worldwide [3, 4]. The Emirates Gastroenterology and Hepatology Society have launched a country wide testing campaign across the UAE in co-operation with Ministry of Health in its attempt to combat the HCV infection in Emirates [5]. The high rate of prevalence of HCV in UAE raises the question about the source of the virus. Some argue that the increase in the prevalence of HCV in UAE was due to viral transmission from expatriate populations. They presumed that expatriate populations were exposed to infection in their countries of origin and not in the host countries [6]**.** High HCV prevalence of 34% among Egyptian blood donors in Saudi Arabia and 11.2% among Egyptian blood donors in Qatar appears to reflect the high HCV prevalence in Egypt [7, 8]**.** The suggested HCV pattern in Gulf Peninsula region was reported for New York City, as HCV prevalence of 15.6% was observed among Egyptian- born individuals living in New York [9].

The hypothesis of that Egyptian are responsible for the increase in HCV prevalence in Gulf Peninsula, including UAE, should be evaluated on experimental basis. In order to test the above hypothesis, there is a need to select specific marker(s) that is sensitive to discriminate between sources of infection whether it is Egyptian or

from Emirates. Molecular epidemiology of HCV genotypes may be the specific approach that able to serve such discrimination.

Hepatitis C is divided into seven distinct genotypes throughout the world with multiple subtypes in each genotype class [10]**.** A genotype is a classification of a virus based on the genetic material in the Ribonucleic acid (RNA) strands of the virus. Generally, patients are only infected with one genotype, but each genotype is actually a mixture of closely-related viruses called quasi-species. These quasi-species have the ability to mutate very quickly and become immune to current treatments, which explains why chronic Hepatitis C is so difficult to treat [11,12].

Differences among HCV genotypes in geographic distributions have provided investigators with an epidemiologic marker that can be used to trace the source of HCV infection in a given population [13]**.** HCV genotype1 may represent a more aggressive strain and one that is less likely to respond to interferon treatment than HCV genotype2 or 3 [12, 14]**.** However, these observations require confirmation before HCV genotyping can be used in clinical settings.

Patients and methods

Study Design

Type: Hospital based descriptive case control study.

Timing: Prospective.

Study population

In order to evaluate the genotype of HCV among the patients who have HCV positive antibodies, this study was carried on 193 patients with HCV positive antibodies and laboratory diagnosis for their genotypes and differentiate them according to their nationality. The study was conducted from June 2013 to December 2017 at the Medical as well as Infectious Diseases clinics at Khalifa Hospital Abu Dhabi, UAE. Khalifa hospital is a tertiary care center and is accredited by the Joint Commission International (JCI). The patients with positive screening test for HCV antibodies referred from other clinics and peripheral health centers were included in the study. The study was designed to include patient's demographics (age, sex, nationality, etc.), clinical information including the various risk factors (I.V. drug abuse, sexual contacts, blood transfusion, operative procedures and tattoo marks) for the transmission of HCV and laboratory data which included serum HCV RNA levels, HCV genotypes and liver function test (LFTs0. The patients were included into the study after informed consent.

A blood sample of each patient was collected and the confirmation of HCV was done by Western blot (HCV Blot 3.0 by MP Diagnostics). The confirmed cases were further tested for HCV RNA levels by polymerase chain reaction (Amplicor HCV Kit, Roche Diagnostic System) and subsequently HCV-RNA positive patients were genotyped by selective hybridization of amplicons to HCV genotype-specific oligonucleotides (Inno-Lipa2, Innogenetics). Liver function test (LFTs) was done by Hitachi Machine 912.

Exclusion Criteria

The patients with positive serology for Hepatitis B virus and Human Immunodeficiency Virus, on haemodialysis, on immunosuppressive drugs and those whose HCV RNA was negative on initial assessment were excluded from the study.

Sampling

Ten milliliters, venous blood, were collected from each case and control by clean vein puncture using disposable plastic syringes. Blood was withdrawn slowly without venous stasis and care was taken to avoid frothing and with minimal delay. Blood samples in plain tubes were put in a water bath at 37° C for complete clotting

for 30 min. All samples were centrifuged at 1500 x g for 20 min. Serum separated was divided into 2 aliquots. The first for liver enzymes, and the second for subsequent PCR assays. They were stored at -70°C till the time of assay. Before performing assays, samples were allowed to come to room temperature (15-28°C) and mixed by gentle swirling or inversion.

HCV antibodies

The nitrocellulose strips contain five recombinant HCV proteins form the capsid, NS3-1, NS3-2, NS4, and NS5 regions of the HCV genome. The HCV proteins are expressed as GST fusion proteins, so a GST control band is included to indicate reactivity to native GST. The blots also contain an IgG control band and an anti-IgG band. Individual nitrocellulose strips are incubated with diluted serum or plasma specimens and controls. Specific antibodies to HCV, if present in the specimen, will bind to the HCV proteins on the strips. The strips are washed to remove unbound materials and then incubated with affinity purified anti-human IgG conjugated with alkaline phosphatase. The conjugate antibody will bind to any antigen-antibody complexes formed on the blots. Unbound conjugate is removed by washing. A BCIP/NBT substrate is added to visualize reactive protein bands on the blots.

The MP Diagnostics (MPD) HCV BLOT 3.0 is a qualitative enzyme immunoassay for the in vitro detection of antibodies to HCV in human serum or plasma. It is intended for use as a more specific supplemental test on specimens found repeatedly reactive using methods such as the ELISA.

HCV RNA PCR

The AMPLICOR HCV Test, v2.0 is based on five major processes: specimen preparation, reverse transcription to generate cDNA from target HCV RNA and HCV Internal Control (HCV IC) RNA, PCR amplification of target cDNAs using HCV-specific primers, hybridization of the amplified cDNAs to target-specific oligonucleotide probes, and colorimetric detection of the probe-bound amplified cDNAs. The AMPLICOR HCV Test, v2.0 permits the simultaneous reverse transcription and PCR amplification of HCV and HCV IC target RNAs.

The Master Mix reagent contains a primer pair that is specific for both HCV and HCV IC RNAs. Detection of amplified DNA is performed using target-specific oligonucleotide probes that permit independent identification of HCV amplicon and HCV IC amplicon. Appropriate selection of primers and probe was critical for AMPLICOR HCV Test, v2.0 detection of all recognized genotypes. Accordingly, selection of the target RNA sequence was based on identifying a region of the HCV genome that was maximally conserved among genotypes. HCV RNA sequences are most conserved in the 5′ untranslated region (UTR). The AMPLICOR HCV Test, v2.0 uses primers KY78 and KY80 to amplify a 5′ UTR sequence of 244 nucleotides. HCV RNA sequence corresponding to these primers and the capture probe are located in the most conserved 5′ UTR domains.

HCV Genotyping

Viral RNA was purified using a QIA amp viral RNA minikit (Qiagen, Hilden, Germany) and was subjected to reverse transcription-PCR (RT-PCR) with a Versant HCV amplification 2.0 kit (manufactured by Innogenetics, Ghent, Belgium, for Siemens, Tarrytown, NY, USA). The 240-bp 5′ UTR and 270-bp core fragments were co-amplified. Subsequently, amplicon denaturation, hybridization, washing, and color development of the genotyping strips were carried out on an Auto-LiPA system (Innogenetics) with a Versant HCV genotype 2.0 kit (Siemens). The results were interpreted according to the LiPA 2.0 interpretation chart, where the line patterns and the corresponding genotyping results are listed.

A line-probe assay (LiPA) employing reverse hybridization of PCR amplicon to type-specific probes on nitrocellulose strips is available commercially (INNO-LiPA HCV II; Innogenetics, Norcross, GA) for HCV genotyping. The LiPA is based on variations found in the 5' UTR of the different HCV genotypes.

Statistical Analysis:

Patients' data were tabulated and processed using SPSS (17.0) statistical package for Windows 7.Quantitative variables were expressed by means and standard deviation and were analyzed using student's unpaired t-test. One-way ANOVA test was used to compare more than two groups as regard a quantitative variable (SD<50% mean). Mann Whitney Willcoxon test was used instead of unpaired t-test in non-parametric data (SD>50% mean).Qualitative data was expressed by frequency and percent and were analyzed using Chi-square. The significance of departure from the null hypothesis is then deduced from special tables, with the degree of freedom corresponding to the actual number of pairs under study. P value <0.05 considered significant.

Results

Part1: Sample Descriptive

Gender.

The frequency distribution of HCV antibodies was significantly higher in male [76.68%] as compared to female [23.32%], Figure1.

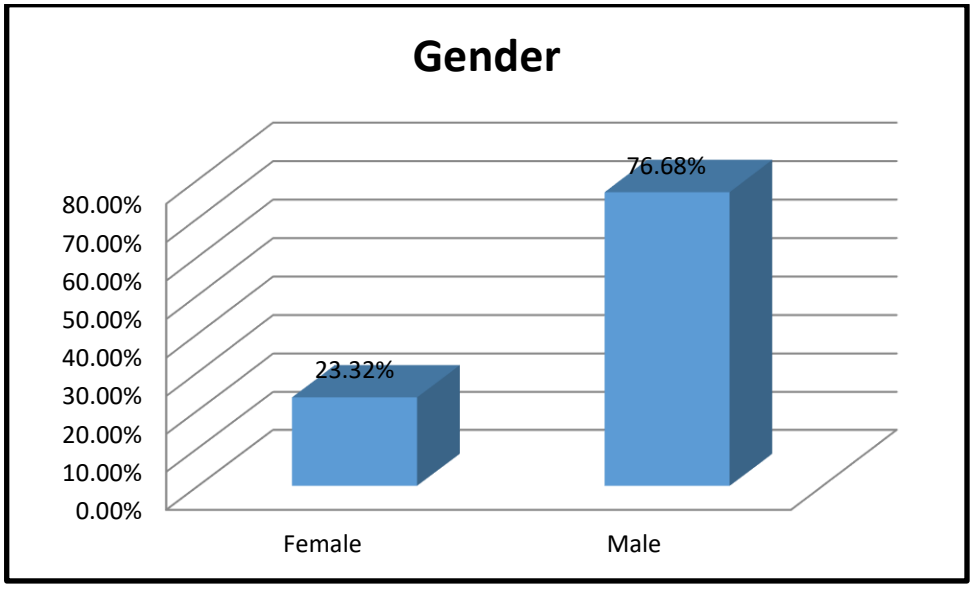

Figure (1) Frequency distribution of the sample according to gender.

Age.

The age range from (24 to 66) year, with a mean of (44.23 ± 9.80) year. The predominant cases were in the age group of (41-45) years, Table1.

Nationality

The frequency distribution of HCV infection was significantly higher in Egyptian expatriate (60.1%), while it forms (18.13%) in Emirati citizen, 21.76% in other nationality, Figure2.

Table (1) Frequency distribution of HCV cases according to age group.

Age group in year	Number	Percent
< 20	0	0%
21 – 25	2	1.6%
26 - 30	12	9.6%
31 – 35	18	14.4%
36 – 40	13	10.4%
41 – 45	25	20.0%
46 – 50	24	19.2%
51 – 55	15	12.0%
56 – 60	13	10.4%
≥ 61	3	2.4%
Total	125	100.0%

- **People with missing in Genotype are excluded from the analysis**

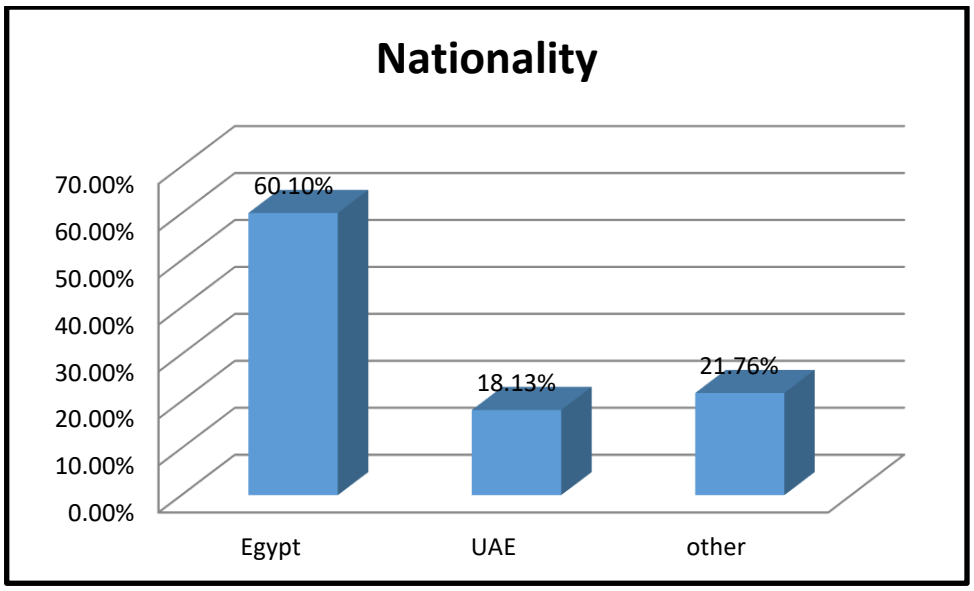

Figure (2) Frequency distribution of the sample according to Nationality

Genotype

The predominant genotype in the studies cohort was type 4 [67.93%], followed by type 1 [16.85%] and type 3 [15.22%], Figure 3.

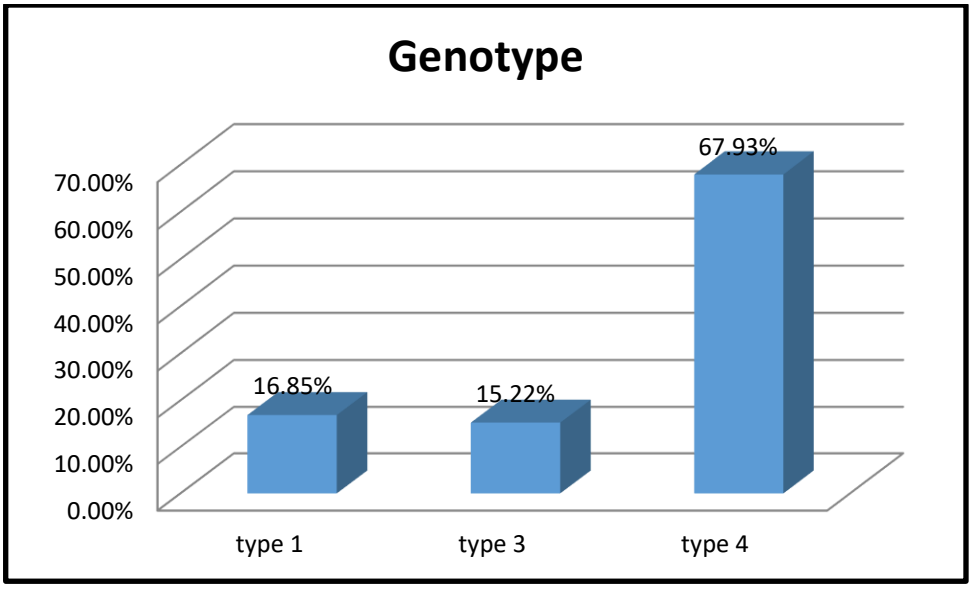

Figure (3) Frequency distribution of the sample according to Genotype

Part 2: Testing the bivariate relations

Studying the relation between Genotype and gender

Type 3 genotype is less common among female compared to type 4 Genotype. The similar pattern can be seen for males also. According to the table (2), the Contingency Coefficient was 0.34, there is significant [P=0.001] moderate relation between gender and genotype. The table shows that type 4 Genotype is the most common for females with percentage of 52.38% compared to 72.54% for males. Also, type 3 Genotype is the least common for females with percentage of 7.14% but type 1 Genotype is the least common for males with percentage of 9.86%, Table (2).

Table (2) Frequency the distribution of Genotype by gender

Genotype	Gender				Total	
	Female		Male			
	Frequency	%	Frequency	%	Frequency	%
1	17	40.48	14	9.86	31	16.85
3	3	7.14	25	17.61	28	15.22
4	22	52.38	103	72.54	125	67.93
Total	42	100.00	142	100.00	184	100.00

- **People with missing in Genotype are excluded from the analysis**
- **The value of the Contingency Coefficient is 0.34 and its P= 0.001**

Studying the relation between Genotype and nationality.

The frequency distribution of Genotype among Egyptian, UAE and other nationalities are shown in Table (3).It is clear that type 4 Genotype is the most common in Egypt as compared to type 1 in UAE, while type 3 and 4 in UAE have similar low rate of spread. According to the above table and the Contingency Coefficient, there is significant strong relation between nationality and Genotype. The

table shows the type 4 Genotype is the most common for Egypt with percentage 97.30% but type 1 Genotype is the most common in UAE with percentage 68.60%. Also, there no type 3 Genotype recorded in the sample among Egyptian people but type 3 Genotype is observed for Emirate patients with percentage 14.30%. Type 3 Genotype has the highest spread rate among other nationalities with percentage 63.90% while type 1 Genotype is the least common with percentage 11.1%.

Table (3) The Frequency distribution of Genotype by nationality

Genotype	Nationality					
	Egypt		UAE		Other	
	Frequency	%	Frequency	%	Frequency	%
1	3	2.65	24	68.57	4	11.11
3	0	0.00	5	14.29	23	63.89
4	110	97.35	6	17.1	9	25.00
Total	113	100.0	35	100.0	36	100.0

- **People with missing values in Genotype are excluded from the analysis**
- **The value of the Contingency Coefficient is 0.7 and its P = 0.0001**

Studying the relation between Genotype and age

Table 4 shows the mean age for different types of virus genotypes.

Table (4) Mean age by genotype

Genotype	Number	Mean age in year
Type 1	31	45.00
Type 3	29	43.57
Type 4	125	44.42
Total	184	44.23

- **People with missing values in genotype are excluded from the analysis**
- **ANOVA F=0.13; P=0.87.**

The mean age is similar for the different genotype viruses with a slightly decline in type 3 Genotype. Analysis of variance (ANOVA) is conducted to compare the differences in the mean of age between the genotype groups and indicated none significant differences [F=0.13; P=0.87].

Part 3: Regression models analysis.

Studying the effect of gender, age and nationality on the virus genotype

Since we concern with the effect of the age, sex and nationality, as explanatory variables, on the virus genotype, as a dependent variable which is a categorical variable, then the best suitable statistical technique to study the effect of the covariates on the dependent variable is the multinomial logistic regression that enables us to use a categorical variable as a dependent variable. The multinomial logistic regression model is constructed by regressing the virus genotype on age, gender and nationality. The likelihood ratio test for testing the significance of the model shows, as in Table (5), that the model is significant as the P-value is less than 0.05 which means that the independent variables has significant effect on genotype .

Table (5) Testing the significance of the model using Chi- square test for Studying the effect of gender, age and nationality on the virus genotype

Chi- square	Degree of freedom	P-value
179.01	6	0.001

Table (6) presents the chi-square test for validating the goodness of fit for the estimated model. The P-value is greater than 0.05 which means that we accept the hypothesis that the model is good fitted.

Table (6) Testing the goodness of using Chi- square test for studying the effect of gender, age and nationality on the virus genotype

Chi- square	Degree of freedom	P-value
165.7	200	0.96

The Pseudo R- square measures the percentage of the variation in the Genotype interpreted by the independent variable. Its value for the estimated model is 0.76 which means the model could interpret 76% of the variation in the dependent variable. By using the multinomial logistic regression, a baseline category for the dependent variable should be specified. In our case, the type (4) is used as a baseline category. Regarding to the parameter estimates, Table7 shows the estimated coefficients besides their P-values. For simplicity, the significant variables only are shown, so the age which has insignificant relation with genotype after excluding the effect of both sex and nationality is omitted from the table.

From table 7, we can notice that the category (female) of sex has a negative significant relation with type 3 genotype after excluding the effect of nationality. The odds ratio is .092 which means that being female, the probability (relative risk) of infecting with type 4 genotype is 11 times the probability (relative risk) of infecting with type 3 genotype after excluding the effect of nationality. In addition, Egypt has a significant negative relation with type 1 genotype compared to a significant positive relation between the UAE and type 1 genotype after excluding the effect of sex. The values of odds for the Egyptian nationality and Emirate nationality is 0.08 and 10.24 respectively, which indicates that being Egyptian instead of (other) nationality, the probability (relative risk) of infecting with type 4 genotype is 11 times the probability (relative risk) of infecting with type 1 genotype whilst being Emirate instead of (other) nationality, the probability (relative risk) of infecting with type 1 genotype is

10 times the probability (relative risk) of infecting with type 4 genotype. This is strong evidence that type 4 genotype is more common for Egyptian citizens than type 1 genotype while type 1 Genotype is more in UAE than type 4 genotype.

Table (7): The estimated parameters of coefficients besides their P-values for studying the effect of sex, age and nationality on the virus Genotype

Genotype		Coefficient	P-value	Odd ratio
Type 1	Intercept	-1.256	0.099	
	Egypt	-2.462	0.006	0.085
	UAE	2.327	0.003	10.246
	Other	Base line		
	Female	0.666	0.314	1.947
	Male	Base line		
Type 3	Intercept	1.712	0.001	
	Egypt	-25.56		<0.0001
	UAE	-1.45	0.06	0.23
	Other	Base line		
	Female	-2.390	0.004	0.092
	Male	Base line		

- **The level of significance is set to 0.05**
- **(type 4) is chosen as a baseline category for Genotype**
- **(other) is chosen as a baseline category for nationality**
- **(male) is chosen as a baseline category for sex**

Regarding to type 3 genotype, the odds ratio for Egypt category is almost zero, this is as there is no observed Egyptian patient with type 3 genotype in the sample. Regarding to UAE category, the rate of infecting with type 3 genotype is similar to the rate of infecting with type 4 genotype after excluding the effect of sex as the P-value of UAE in type 3 genotype is .23 (greater than 0.05). This is evidence that type 3 genotype is not common in Egypt but type 3 genotype and type 4 genotype have an existence in UAE, actually lower to type 1 genotype which is the most common.

Discussion

The global epidemiology of HCV varies greatly all over the world, and different studies have shown such variations. Hepatitis C virus was found to be endemic in certain countries, and different studies have confirmed such endemicity [15]. It is important to consider the short coming of regional studies is in Arab world. Most of the epidemiological studies carried out individually based upon seroprevalence of HCV among specific groups. These include blood donors, heath care workers, or patients undergoing haemodialysis. Such studies were not representative of the community; they were usually carried out by independent scientists. Even though, some countries lack such studies [16].

The prevalence of HCV genotypes varies geographically. HCV genotype 1, 2 and 3 are distributed worldwide and their relative prevalence varies from one

geographic area to another, whereas genotype 4 is predominantly prevalent in the Middle East and Africa, genotype5 in South Africa and genotype6 in Southeast Asia [17], but there is lack of comprehensive data to make the appropriate inference as to which genotype is more common in these countries. According to the recent studies, genotype4 is predominant in Egypt, 4 and 1 in Kuwait, Saudi Arabia and Syria, and genotype1 in Lebanon, Iraq and Iran [18, 19]. The knowledge of distribution of HCV virus is important not only in knowing the prevalence of different genotypes, possible routes of transmission, to define the period and aspect response to the anti HCV therapy but also for future vaccine development.

Understanding of the geographic distribution of common genotypes requires detailed knowledge about the routes of transmission, prevalence of HCV in general population and in the various high risk groups, and also phylogenic evolution of types and subtypes over a long period [20]. Unfortunately, there is little information available on these topics, particularly from Middle East countries and more specifically from United Arab Emirates; however there are few studies which have reported high prevalence of genotype 4 in these countries [21].

Pacsa et al., [22] have reported the common genotype in the Republic of Yemen, Kuwait, Iraq and Saudi Arabia is genotype 4. Genotype 4 is also reported by McOmish et al., [23] and Ray et al., [24] as a most common genotype in Syria and Egypt respectively.

Messina et al., [25] in a review that included 1217 studies that were published between 1989 to 2013 reported that HCV genotype1 is the most prevalent globally, comprising 46.2% of all HCV cases, about 1/3 of which in East Asia. Genotype 3 is the next common prevalent worldwide and form 30.1%, while genotype2, 4, and 6 are responsible for a total 22.8% of all cases, genotype 5 forming <1%. In addition, type 1 and 3 dominate in most countries irrespective of economic status, in contrast type 4 and 5 are predominant in lower- income countries. This finding was different from the studying of Messina et.al. [25], where type 4 was the predominant accounting 67.93% of cases, while type 1 forming 16.85% and type 3 forming 15.22%. Also in Yemen, Al- Nabehi et al., [26], found that HCV genotype 4 and 1a were the most common genotypes in urban Yemeni population.

Two recent systematic review and meta- analysis [2, 27], on the prevalence of HCV genotype in Eastern Mediterranean Regional Office of WHO (EMRO) and Middle Eastern countries were published. Sadeghi et al [27], included 67681 HCV-infected individuals from 134 studies, found that subtype 1a was the predominant in Iran [42%], followed by subtype 3a [35%]; in Pakistan 3a [56%] was the common; in Saudi Arabia and Egypt, genotype4 the predominant with a rate of 65% and 69% respectively. In Tunisia and Morocco, subtype 1b was the most common subtype with a rate of 69% and 32% respectively. They concluded that genotype 1 and 3 were the common type in Iran and Pakistan, while genotype 4 and 1 were the predominant type in Middle East Arab countries.

In a systematic review and meta-analysis included 60,319 of patients infected with HCV from 187 studies and reported that in Turkey, Cyprus, and Iran HCV genotype1 was the predominant with rates of 82%, 68% and 55% respectively. While in Egypt, Iraq, Saudi Arabia and Syria, type 4 was the predominant genotype with rates of 86%, 60%, 56% and 57% respectively. They concluded that genotype 4 was the prevalent genotype in Middle East region, with a rate of 74.7%, followed by genotype1 (15.1%). In addition, genotype4 was predominant in Arab countries, while type 1 was the predominant in non-Arab countries in the Middle East region, such as

Iran, Turkey, and Cyprus. The present study finding was in the line of these two reviews as the predominant genotype was type 4[2].

Khdeir et al [28], Baghdad, Iraq, performed a study during the period from 21/9/2014 to 23/9/2015 found that HCV genotype frequency was 1a, 1b, 2 and 3a in 37.25%, 17.65%, 4.9% and 0.98% respectively, indicating that genotype 1 was the dominant type [54.9%]. Mixed genotypes found in 14.7% of their studied samples and mostly a combination of type 1 and 3a [11.76%] [28]. In contrast, other study from Baghdad, Iraq [2012-2013] performed on haemodialysis patients shows that HCV genotype4 was found the predominant [56%], followed by 1b [23%], 1 a [12%] and 3 [9%][29]. In addition, other study performed in Mosul, Iraq, which included thalassemic patients, chronic liver disease, and blood donors report that genotype4 was the dominant in thalassemia [94%], chronic liver disease [85%] and blood donors [80%] [30].

In a systematic review and meta-analysis which included 159 studies in the Arabian Gulf countries reported that HCV prevalence among nationals was 0.24% in the United Arab Emirates (UAE), 0.44% in Kuwait, 0.51% in Qatar, and 1.65% in Saudi Arabia. No data were available for Bahrain or Oman. Among the entire resident populations, HCV prevalence was 0.30% in Bahrain, 0.41% in Oman, 1.06% in Qatar, 1.45% in Kuwait, 1.63% in Saudi Arabia, and 1.64% in UAE. A higher prevalence was observed among expatriate populations such as Egyptians. Among the high-risk populations, HCV prevalence was as high as 78.6% in the multi-transfused and 74.6% in people who inject drugs. They concluded that National-level HCV prevalence in the Arabian Gulf region is comparable to global levels. A higher prevalence is found in specific expatriate populations, reflecting the prevalence in their countries of origin. Most exposures appear to occur in high-risk groups and these are often linked to medical care [6].

The prevalence of HCV in Arab countries nowadays with the lack of immediate and effective intervention illustrates the possibility of tremendous incidence increase in the next two decades. In few countries of the Middle East and North Africa (MENA) region, namely Egypt and Pakistan, HCV is prevalent at high levels: 14.7% in Egypt [31] and 4.8% in Pakistan [32]. Meanwhile, infection levels remain poorly estimated for most MENA countries including those in the Fertile Crescent (FC) [33].While some of the prevalent HCV exposures may date to times before the enforcement of more stringent blood screening and infection control protocols in these countries, the totality of the evidence supports the conclusion of ongoing transmission in some clinical settings and through medical procedures. These results emphasize the importance of reinforcing blood screening and infection control in health care facilities. Other HCV risk factors identified in this study included exposure to infected needles and sharp objects during tattooing and cupping procedures [34].The latter, also known as hijama, is a common practice by community healers in MENA [35].

There is also evidence elsewhere in MENA of a wider scope of community related HCV exposures as well as traditions of some professions, such as barbers, administering health care related procedures such as injections [36].These findings highlight the relevance of controlling these exposures and insuring the safety of injections. This could be done, for example, by wide-scale replacement of current re-usable syringes with safety-engineered “smart” syringes [37].

Migration dynamics within MENA may have played a role in HCV epidemiology in this sub region. This is specially so in relation to the epidemic in Egypt, the country with the largest HCV prevalence worldwide [31].The epidemic in Egypt is almost exclusively dominated by genotype4 (>90%) [38].Genotype4 in

MENA appears to have a larger frequency in countries geographically close to Egypt and/or in countries that host or have hosted large migrant labor populations from Egypt [39]. Movement of Egyptian labor, who often came from rural areas most affected by HCV, may have contributed to higher circulation of genotype4 in host countries, but has had a limited impact on HCV prevalence in these countries [39].

In UAE, increased prevalence of HCV among spouses was detected with longer duration of marriage being an important risk factor; such infection was passed to children at an early stage [40].A molecular study was conducted to investigate the prevalence of Hepatitis C virus genotypes in HCV infected population of UAE. 67 HCV seropositive samples were collected from different health care centers. Quantitative analysis of these samples using PCR resulted in 67 positive samples. HCV genotype 4 was the predominant genotype (46.2%) followed by genotype 3a (23.8%) and 1a (15%). The predominant genotype among the female patients was genotype4 (65.6%), while genotype3a was the predominant among the male patients (42.8%). The predominance of HCV genotype4 in our population confirms the predominance of HCV genotype4 in UAE and most of the Arab countries in the Middle East [41].

In order to evaluate the genotype of HCV among the patients who have HCV positive antibodies, this study was carried on 193 patients with HCV positive antibodies and laboratory diagnosis for their genotypes and differentiate them according to their nationality.

The present study indicated that HCV infection was significantly higher in Egyptian expatriate with frequency of 60.10% (116 cases out of 193 cases), while it was 18.13% (35 cases out of 193 cases) in Emirati citizen. This finding could be lead to suggest that the Egyptian expatriate may act as source of infection. However, the genotype frequency distribution indicated that HCV infection in Emirati was with significant association with type 1 [OR=10.246; p=0.003], while OR was 0.085 in Egyptian and thus the hypothesis that presumed the increase in prevalence of HCV in Emirati was excluded. In addition, type 3 genotype was with 0% frequency in Egyptian, while it forms 63.89% as a cause of HCV infection in other nationality and thus this finding exclude that Egyptian were the reservoir of infection to other nationality.

Abro et al., [19] found that in contrary to the UAE male patients, UAE female patients had predominantly genotype4 which could be explained by the fact that most of the female patients were of Egyptian origin where genotype4 is the most common genotype and they might have been infected in their country of origin.

In current study, the type 4 genotype is the most common for females with percentage 52.38% compared to 72.54% for males. Also, type 3 genotype is the least common for females with percentage 7.14% but type 1 genotype is the least common for males with percentage 9.86%. Abro et al [19] reported an important finding which was high prevalence of genotype 3 and 1 in IDUs which is similar to IDUs in Europe and USA as reported by Pawlotsky et al., [42]. In the present study, we observed high prevalence of genotype3 and 1 in younger (<40 years) male patients as compared to the older (>40 years) and female patients who predominantly had genotype4 and 1. The above observation is inconstant with the reports of Kabir et al., and Montalvo et al., [43, 44], who found no difference in genotypes in terms of age and sex of the patients. In comparison to genotype3, high prevalence of genotype1 is reported among the patients who received blood/blood products transfusion [45].

The present study found that HCV infection more frequent in male [76.6%] as compared to female [23.32%]. Khdeir et al., [28], found that HCV infection was 48%

in male and 52% in female. Alsamarai et al, [46], study shows that seroprevalence of Anti-HC Ab was not significantly different in female as compared to male for 2011 and 2012. However, Anti-HC Ab seroprevalence was significantly higher in female (1.44%) as compared to male (0.57%). When the data of the 3 years pooled together, the Anti-HC Ab seroprevalence was significantly higher in female (0.72%) as compared to male (0.41%), with male to female ratio of 0.57:1. However, Ataalla et al., [47], Iraq, found higher prevalence of Anti-HCV in male (0.5%) than in female (0.3%) but the difference was not statistically significant in national population. These findings not agreed to that reported by others in Iran [48], Pakistan in blood donors, and India in outpatients' clinic visitors [49] and USA blood donors [50] as the prevalence was higher in male than female. In Egypt, higher HCV prevalence was detected in males compared to females among village residents [51] and blood donors [52]. In Pakistan, although the probability trends were slightly higher in males of all age groups than in females, the differences not statistically significant [53, 54].In a nationwide survey in Poland did not indicated a significant effect of gender on HCV prevalence [34, 55], in Baghdad, Iraq, found a positive association between male gender and HCV infection. However, female was considered with high rate of viral clearance [40%] as compared to male [19%] [56].The variation in gender prevalence rate was mainly attributed to study population as there was a difference between prevalence in general population and risk factor groups. Thus the comparison and interpretation should be performed with caution.

This study shows that HCV infection was higher in age group with a mean age of 44.23 ± 9.80 years, indicating a trend of HCV infections in the age group >41years which indicate that the incidence of HCV increase gradually with the age. This finding was in contrast to that reported for Iraq, as the mean age of their patients was 28.77±13.58 years indicating a trend of HCV infections in younger age groups. However, Khaleel et al., [34] reported a positive increase of anti-HCV antibodies with older age.

In a recent study in Iraq [46], the overall seroprevalence of Anti-HC Ab was significantly different between age groups, with higher prevalence (1.62%) in the age group >45 years, followed by age group of 1-4 years (1.22%). In addition, the same patterns were demonstrated for 2013 and 2012 but not 2011. Furthermore, there was a significant difference in Anti-HC Ab between the study years in both age groups of 15-45 and >45 years. Thus our results indicated that HCV seroprevalence peak in 1-4 years (1.22%) then decline and peak again in >45 years (1.62%). In a national Iraqi population survey, anti-HCV seroprevalence was 0.3% in age 0f 1-10 years and increased gradually with age to pear at of >40 years [47].

Rotermann et al., (2013) [57]in a review for 2009 to 2011 in Canada reported that HCV seroprevalence was higher in age of 50 to 79 as compared 14 to 49 years. In Egypt, the HCV prevalence increased dramatically with age with the highest rates observed among populations aged >40 years [31]. HCV prevalence of 4% to 7% was reported among Egyptian children from rural school children and attending outpatient's clinics [54, 58]. In a prospective study, maximum seropositivity of IgM anti HCV was in 10-20 years of age group of suspected cases of hepatitis and in 0-10 years of controls [59].

HCV prevalence was highest in the age group of 20-29 and 30 -39 years among general population of Lahore, Pakistan [53]. In contrast to our findings HCV prevalence decreased in individuals with age of >40 years [60-62]. However, our study HCV prevalence was agreed to that reported by others [49]. The differences in HCV age related prevalence in different reported studies may be due to influence of

the exposure to different risk factors, religious and social behaviors in different communities. For example, in Australasia, the estimated peak prevalence at ages 20-24 years may reflect the high incidence of HCV among persons who use injecting drugs reported recently [63,64]. Mother-to-infant transmission is the commonest route of transmission in children, with a vertical transmission rate of 5% [65]. Thus the higher HCV seroprevalence in the ages of 1-4 years in our study population may be reflect of high rate vertical transmission and an indicator of high infection in pregnant women. This finding could ascertain the need for a screening of pregnant women in antenatal period. This screening program is cost-effective, since the cost of HCV case treatment is more expensive [$15000] than performing HCV screening for > 6000 samples [1].

The present study indicated a higher HCV seroprevalence in older age group (>45 years). This HCV seroprevalence in old age groups agreed to that reported in a hospital based retrospective and community based studies in Iraq [46,66], however, it is lower to the mean of the prevalence in North Africa and Middle East [67,68]. Those are in adult age when the HCV screening programs are founded in Iraq [1995], which means they are not included in the screening program and thus with increasing age the prevalence increased due to both vertical and horizontal infections. In addition, during this period Iraq was under economic sanction and after the American occupation of Iraq, which disrupt the health care system. The shortage of materials and equipment for blood screening lead to that blood and blood products given to patients without screening [69]. Furthermore, the possibility of transmission via tattooing, piercing, Hijama, and health care settings due to that these practices conducted by unsafe healthy practices. Sexual transmission also may be implicated as cause that leads to high rate of HCV transmission because of the loss of monitoring program for HCV in prostitutes.

Shakeri et al., [48] from Iran, found that HCV prevalence was higher in those with age of 50 – 59 years; however, the mean age of their population study was 39 years. Prevalence of HCV peak at 55-64 in Central, East and Southern sub-Saharan Africa; Americas; Eastern Mediterranean; Asia; and Eastern, and Western, Europe; however, West sub-Saharan African the curve have two peaks, 1st at 15-19 years and 2nd at 55-64 years. In Western Pacific the pattern of seroprevalence across age in Australasia exhibit a rapid increase in prevalence peaking 1st at 20-24 years and later in 55-64 years age. In addition, in Central Europe an early peak in ages 1-4 years is seen in Central Europe [4].

Multinomial Logistic regression line analysis indicated that age, gender and nationality were independent variables with significant effect on genotype. Female gender was with negative significant relation with genotype3 after excluding the effect of nationality and female are susceptible 11 times to be infected with genotype 4 that the infection for genotype 3. In addition, Egyptian has a significant negative correlation with type 1, while UAE are with significant positive correlation with genotype1 after excluding the effect of gender. Furthermore, Egyptian is 11 times susceptible for genotype4 than for genotype1, while the Emirati are 10 times susceptible for genotype1 than for genotype4. These finding is strong evidence that exclude Egyptian as a cause for increase of hepatitis C in Emirati.

Egyptian are not susceptible for HCV genotype3 as shown by logistic regression line analysis [OR=0], while in UAE, the rate of infection with type 3 was similar to their infection rate with type 4 after excluding the effect of gender. Thus genotype3 is not common in Egyptian, however, type 3 and 4 are existed in UAE, but with lower incidence to that of type 1, which is the most common. From these data we conclude

that nationality is an important risk factor that influence infection genotype and subsequently influence the disease severity, response to treatment and that influence infection genotype and subsequently influence the disease severity, response to treatment and prognosis.

The impact of HCV on health and medical care in Arabian countries is a major problem for the community and infectious disease physicians as this study illustrate evolving of new epidemiological characteristics. There are insufficient data about HCV prevalence and prevention methods in most of the Arab countries region [16]. Most of the information about the prevalence of HCV infections has generally been limited to laboratory data and personal interest of research projects in certain education institutes. There is no national routine surveillance which represents the sound data source in the Arabian community. To establish public health strategies, more well-programmed, population based and certain HCV infections at risk survey are needed in Iraqi community. Thus because of such problem, a national screening community based program on the basis of regional and sub-regional sampling are warranted. In addition, most of the HCV infection survey used serological methods that detect antibodies in their screening, and none seroconverted HCV infection is not detected. Thus this screening approach may not detect some of cases who are not seroconverted. So in order to apply more sensitive and specific screening programs we must detect HCV antigens rather than antibodies. The present study used ELISA for detection of HCV antibodies and PCR to detect virus particle, giving it high sensitivity and specificity and diagnosis predictively.

In conclusion, HCV more predominant in Egyptian than in UAE. However, Egyptian has a significant negative correlation [OR=0.085, P=0.006] with type 1, while UAE are with significant positive correlation with genotype 1 after excluding the effect of gender. Furthermore, Egyptian are 11 times susceptible for genotype 4 than for genotype 1, while the Emirati are 10 times susceptible for genotype 1 than for genotype 4. However, the genotype frequency distribution indicated that HCV infection in Emirati was with significant association with type 1 (OR=10.246; p=0.003), while OR was 0.085 in Egyptian and thus the hypothesis that presumed the increase in prevalence of HCV in Emirati was excluded. In addition, type 3 genotype was with 0% frequency in Egyptian, while it forms 63.89% as a cause of HCV infection in other nationality and thus this finding is strong evidence that exclude Egyptian a cause for increase of hepatitis C in Emirati.

Recommendations:-

Health care setting need to implement and address primary prevention programs and a grassroots programs and support is an essential in control and prevention of HCV infections in health setting in all Arabian countries.

Reference

1. World Health Organization. The growing threat of hepatitis B and C in the Eastern Mediterranean region: a call for action. Regional office for the Eastern Mediterranean. EM/RC56/3. 2009.
2. Ghaderi-Zefreh H, Gholami-Fesharaki M, Sharafi H, Sadeghi F, Alavian S.M. Distribution of Hepatitis C Virus Genotypes in Middle Eastern Countries: A Systematic Review and Meta-Analysis. Hepatitis Mon. 2016; 16(9): e40357.
3. Lavanchy D. The global burden of hepatitis C. Liver Int, 2009; 29 (Suppl 1):74-81.

4. MohdHanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age specific antibody to HCV seroprevalence. Hepatology, 2013, 57(4):1333–42.
5. http://www.natap.org/2015/HCV/100215 03.htm
6. Mohamoud YA, Riome S, and Abu-Raddad LJ. Epidemiology of hepatitis C virus in the Arabian Gulf countries: Systematic review and meta-analysis of prevalence. Nat J Infect Dis. 2016 May; 46:116-25.
7. Fawzi Z, Al Hilali A, Al Maliki A, Al Matawa H, Yousef B, Ali Bin Ali A, Al Mansour S. Survey of hepatitis markers among donors in the state of Qatar. Qatar Med J 2007; 16:47-50.
8. Omar AS, Zuebi FE. Disease markers in blood donors at King Fahad hospital, Al Baha. Ann Saudi Med 1996; 16:37-41.
9. Perumalswami PV, Dewolfe MF, Orabee H, Regab A, Adams M, Kapelusznik L, et al. Hepatitis screening beyond CDC guidelines in an Egyptian immigrant community. Liver Int 2014; 34:253-8.
10. Smith DB, Buck J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment Web resource. Hepatology 2014; 59:318-327.
11. Palmer M. Dr. Melissa Palmer's Guide to Hepatitis and Liver Disease. Revised Edition, New York: Avery Trade, 2004.
12. Medscape. Diagnosis and Management of Acute Liver Failure. http://www.medscap.com/viewarticle/720697_3.
13. Power J P, Lawlor E, Davidson F, Holmes E C, Yap P L, Simmonds P. Molecular epidemiology of an outbreak of infection with hepatitis C virus in recipients of anti-D immunoglobulin. Lancet. 1995; 345:1211–1213.
14. Zein N , Rakela J, Krawitt E L, Reddy K R, Tominaga T, Persing D H. The Collaborative Study Group. Hepatitis C virus genotypes in the United States: epidemiology, pathogenicity, and response to interferon therapy. Ann Intern Med. 1996; 125:634–639.
15. Sievert W, Altarif I, Razavi HA, Abdo A, Ahmed EA, Alomair A. A systematic review of hepatitis C virus epidemiology in Asia, Australia and Egypt. Liver Int 2011; 31:61-80.
16. Daw MA, Dau AA. Hepatitis C virus in Arab world: a state of concern. Scientific World Journal. 2012; 2012:719494. doi: 10.1100/2012/719494..
17. Zein NN. Clinical significance of hepatitis C virus genotypes. Clin Microbiol Rev; 2000:13: 223-232.
18. Osaba AO. Hepatitis C virus genotypes in Saudi Arabia. Saudi Med J.2000; 23: 7-12.
19. Abro AH, Al-Dabal L, Younis NJ. Distribution of Hepatitis C virus genotypes in Dubai, United Arab Emirates. J. Pak Med Assoc. 2010; 60(12):987-990.
20. Zali MR, Mayumi M, Rouf M, Noowroozi A. Hepatitis C virus genotypes in the Islamic Republic of Iran: a preliminary study. Eastern Mediterr Health J. 2000 ; 6: 372-7.
21. Das BR, Kundu B, Khandapkar R. Geographical distribution of hepatitis C virus genotypes in India. Indian J Pathol Microbiol; 2002; 45: 323-8.
22. Pacsa AS, Al-Mufti S, Chugh TD, Said-Adi G. Genotypes of Hepatitis C virus in Kuwait. Med Principles Pract; 2001; 10: 55-7.

23. McOmish F, Yap P L, Dow BC. Geographical distribution of hepatitis C virus genotypes in blood donors and international collaborative survey. Journal Clin Micro 1994, 32: 884-892.
24. Ray SC, Arthur RR, Carella A, Bukh J, Thomas DL. Genetic Epidemiology of hepatitis C throughout Egypt. J Infect Dis. 2000; 182; 239-44.
25. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2015; 61(1):77-87.
26. Al-Nabehi BAH, Al-Ahamahy H, Saeed WSE, Musa AM, El Hassan AM, Khalil EAG. Sero-Molecular Epidemiology and Risk Factors of Viral Hepatitis in Urban Yemen. Int J Virol 2015, 11: 133-138.
27. Sadeghi F, Salehi-Vaziri M, Almasi-Hashiani A, Gholami-Fesharaki M, Pakzad R, Alavian SM. Prevalence of Hepatitis C Virus Genotypes among Patients in Countries of the Eastern Mediterranean Regional Office of WHO (EMRO): A Systematic Review and Meta-Analysis. Hepatitis Mon. 2016; 16(4): e35558.
28. Khdeir NA, Al-Hmudi HA, Alhajim D. Genotype of hepatitis C virus in patients of Basrah Province/Iraq. Int J Inn Res Sci Eng Tech 2016; 5:10363-68.
29.Manuti JK, Al Obeidy ES, Abdul Rasheed O. Distribution of HCV genotype among haemodialysis patients. Int J Advance Res 2014; 2:4-8.
30. Khalid MD, Abdulla BA. Hepatitis C virus genotype in Iraq. Iraqi J Biotech 2012; 11: 475-480.
31.Mohamoud YA, Mumtaz GR, Riome S, Miller D, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Egypt: a systematic review and data synthesis. BMC infectious diseases 2013; 13:288.
32.Umar M, Bushra HT, Ahmad M, Data A, Ahmad M, Khurram M, et al. Hepatitis C in Pakistan: a review of available data. Hepatitis monthly, 2010; 10 (3):205–14.
33. Chemaitelly H, Chaabna K, Abu-Raddad LJ. The Epidemiology of Hepatitis C Virus in the Fertile Crescent: Systematic Review and Meta-Analysis 2015;PLoS ONE 10(8): e0135281.
34. Khaleel HA, Turky AM, Al-Naaimi AS, Jalil RW, Mekhlef OA, Abdul Kareem S, et al. Prevalence of HBsAg and anti HCV Ab among patients with suspected acute viral hepatitis in Baghdad, Iraq in 2010. Epidemiology Reports 2014; 1(1).
35. El-Ghitany EM, Abdel Wahab MM, Abd El-Wahab EW, Hassouna S, Farghaly AG. A comprehensive hepatitis C virus risk factors meta-analysis (1989–2013); Do they differ in Egypt? Liver international: 2015 Feb; 35(2):489-501. DOI: 10.1111/liv.12617.
36. Mohamoud YA, Miller FD, Abu-Raddad L.J. Potential for human immunodeficiency virus parenteral transmission in the Middle East and North Africa: an analysis using hepatitis C virus as a proxy biomarker. World J Gastroenterol 2014: WJG; 20(36):12734–52.
37. World Health Organization. WHO guideline on the use of safety-engineered syringes for intramuscular, intradermal and subcutaneous injections in health-care settings. 2015 WHO/HIS/SDS/2015.5.
38. Ramia S, Eid-Fares J. Distribution of hepatitis C virus genotypes in the Middle East. Inter J Infect Dis2006; 10 (4):272–7.
39. Fadlalla FA, Mohamoud YA, Mumtaz GR, Abu-Raddad LJ. The epidemiology of hepatitis C virus in the Maghreb region: systematic review and meta-analyses. PLOS ONE 2015;10 (3):e0121873.
40. Kumar RM, Shahul S. Role of breast-feeding in transmission of hepatitis C virus to infants of HCV-infected mothers. J Hepatol 1998; 29(2):191–197.

41. Alfaresi MS. Prevalence of hepatitis C virus (HCV) genotypes among positive UAE patients. Molecular Biol Reports 2011; 38(4):2719.
42. Pawlotsky JM, Tsakiris L, Roudot-Thoraval F, Pellet C, Stuyver L, Duval J, et al. Relationship between hepatitis C virus genotypes and sources of infection in patients with chronic hepatitis C. J Infect Dis.1995; 171: 1607-10.
43. Kabir A, Alavian SM, Keyvani H. Distribution of hepatitis C in the Middle East. Int J Infect Dis. 2006; 10: 272-7.
44. Montalvo BMG, Colorado PLG. Distribution of hepatitis C virus genotypes, risk factors and liver disease in patients from Yucantan, Mexico. Ann Hepatol 2008; 7: 345-9.
45. Roman F, Hawotte K, Struck D, Ternes AM, Servais JY, Ardent V, et al. Hepatitis C virus genotypes distribution and transmission risk factors in Luxembourg from 1991 to 2006.World J Gastroentrol 2008; 14: 1237-43.
46. Alsamarai AGM, Alsamarai G, Fatah A. Seroprevalence of HCV in Iraqi population. Juniper Online J Immuno Virology. 2016;1(2).DOI: 10.19080/JOJIV.2016.01.555565.
47. Ataallah MT, Kaleel AH, Kadoori SM, AL Aanni AS. Prevalence of Hepatitis B and C among blood donors attending the National Blood Transfusion Center in Baghdad from 2006-2009.Saudi Med J , 2011; 32(10):1046-1050 .
48. Shakeri MT, Nomani H, Ghayour Mobarhan M, Sima HR, Gerayli S, Shahbazi S, Rostami S, Meshkat Z. The prevalence of Hepatitis C virus in Mashhad, Iran: a population-based study. Hepatitis Mon. 2013 Mar 12; 13(3):e7723.
49. Sood S, Malvankar S. Seroprevalence of Hepatitis B surface antigen, antibodies to the Hepatitis C virus, and human immunodeficiency virus in a hospital-based population in Jaipur, Rajasthan. Indian J. Community Med. 2010; 35(1):165-169.
50. Sheikh MY, Atla PR, Ameer A, Sadiq H, Sadler PC. Seroprevalence Hepatitis B and C infections among healthy volunteer blood donors in the central California Valley. Gut and Liver, 2013; 7(1):66-73.
51. Sharaf-Eldin S, Salama K, Eldemedresh S, Hassan HMS, Semsem M. Hepatitis B and C viruses in Egyptian children with malignancy. J Med Sci, 2007; 7(6):1003-1008.
52. Rushdy O, Moftah F, Zakarya S. Transmitted transfused viral infection among blood donors during years 2006 and 2007 in Suez Canal area Egypt. Vax Sang, 2009; 96:86-87.
53. Anwar MI, Rahman M, Hassan MU, Iqbal M. The prevalence of Hepatitis C virus infections among general public of Lahour, Pakistan. Virology J. 2013; 10:351
54. El-Raziky MS, El-Hawary M, Esmat G, Abouzied AM, El-Koofy N, Mohsen N, et al. Prevalence and risk factors of asymptomatic Hepatitis C virus infection in Egyptian children. World J Gastroenterol 2007;13(12):1828-32.
55. Hartleb M, Gutkowsky K, Zejda KE, Chudek J, Wicek A. Serological prevalence of Hepatitis B virus and C virus infection in the elderly populating: Polish nationwide survey – PolSenior. Eur J Gastroenterol Hepatol. 2012; 24(11):1288-1295.
56. Micallef JM, Kaldor JM, Dore GJ. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. J Viral Hepat 2006; 13:34-41.
57. Rotermann M, Langlois K, Andonov A, Trubnikov M. Seroprevalence of Hepatitis B and C virus infections: results from the 2007-2009 to 2011 Canadian

health measures survey. Statistic Canada, Catalogue no.82-003-X.Health Reports, 2013; 24(11):3-13.
58. El Sherbini A, Mohsen SA, Seleem Z, Ghany AA, Moneib A, Abaza AH. Hepatitis B virus among schoolchildren in an endemic area in Egypt over a decade: impact of hepatitis B vaccine. Am J Infect Control 2006; 34: 600–2.
59.Rajani M, Jais M. Age-wise seroprevalence Hepatitis C virus infection in clinically suspected infectious Hepatitis patients attending a tertiary care hospital in Delhi. Intern J Med Public Health. 2014; 4(1):78-81.
60. Ahmed W, Jaz B, Javid FT, Jahan S, Shahid M, Khan FM, et al. HCV genotype distribution and possible transmission risks in Lahore, Pakistan. World J Gastroenterol. 2010;16(34): 4321–4328.
61. Muhammad Idrees I, Ahmed H, ul-Ghafoor S, Ali M, Ali L, Ahmed A. Hepatitis C virus genotypes circulating in district Swat of Khyber Pakhtoonkhaw, Pakistan. Virol. J 2011; 8:16.https://doi.org/10.1186/1743-422X-8-16
62. Masood Z, Jawaid M, Khan RA, Rehman S. Screening for Hepatitis B and C a routine preoperative investigations. Park J Med Sci, 2011; 21:455-459.
63. Maher L, Li J, Jalaludin B, Chant KG, Kaldor JM. Big Hepatitis C incidence in new injecting drug users: a policy failure. Aust N Z J Public Health.2007; 31:30-35.
64. Iversen J, Wand H, Gonnermann A, Maher L. Gender differences in Hepatitis C antibody prevalence and risk behaviors amongst people who inject drugs in Australia 1998-2008. Int J Drug Policy.2010; 21: 471-476.
65. McIntyre PG, Tosh K, McGuire W. Caesarean section versus vaginal delivery for preventing mother to infant Hepatitis C virus transmission. Cochrane Database Syst Revs. 2006:CD005546.
66. Ataallah MT, Akram W, AL-Naaimi AS, Omer AR. Epidemiology of viral hepatitis B and C in Iraq: a national survey 2005-2006. Zanco J Med Sci, 2013; 17:370-380.
67.Karoney MJ, Siika AM. Hepatitis C virus (HCV) infection in Africa: a review. Pan African Medical J. 2013; 14:44.2199.
68.World Gastroenterology Organisation Global Guidelines. Diagnosis, Management and Prevention of Hepatitis C. 2013; 6-8.
69.Alawady NB. The prevalence of Hepatitis C virus infections among Iraqi patients registered to Babylon center for inherited blood disorders. Int J Advan Res. 2014; 2(1):383-388.