Prevalence and Risk Factors for Transmission and Genotype of HCV in Iraqi Patients

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Abstract

Background: Hepatitis C virus (HCV) infection is a serious public health problem in the world, and about 170 million patients are chronically infected with HCV. Worldwide; different epidemiologic patterns of HCV infection have been identified.

Aim of the Study: By this study, we try to know the prevalence and risk factors for HCV transmission in Iraqi patients and genotype distribution of the disease.

Patients and Methods: In a cross sectional study of 355898 persons, 232645 males & 123253 females; had been enrolled for the study in Baghdad teaching hospital, Iraqi blood bank, Al-Yarmouk teaching hospital, Al-Nu'man teaching Hospital and Al-Basrah teaching hospital from February 2017 to February 2019. Positive patients for hepatitis C antibody were further studied for way of transmission and viral load and genotype. History had been taken about the possible risk factor for HCV transmission which included (15) risk factors.

Results: A total of 355898 persons, 232645 males & 123253 females their Mean age at diagnosis was 47.3 (\pm 15.6) years, Ranged (19–78) years. The mean body mass index (BMI) of the study sample was 22.9 (\pm 3) kg/m2, studied all screen for hepatitis C antibody 1155 discovered to be positive 650 were males and 505 were females, further study done for viral load in 746 patients and genotype in 633 patients: 356 (48%) with high viral load, 390 (52%) with low viral load and 345 (52.8%) with genotype V and 301 (46.2%) with genotype IV, while genotype II was 2 (0.3%) and III 7 (0.5%), The most common possible ways of transmission of the disease by history in this study was blood transfusion 53%, dental procedure 42%, hemodialysis 36%, caesarean section 34.5%, while injured male during shaving 33%, patient who have surgical operation 24%, Tattooing in 8%, cupping in 6.6%, IV injection in 4% heterosexual seen in 2%, renal transplant in 2%, and only 1% in needle stick injury. while the more frequent genotype is 1 then 4. Hemodialysis and improper use of IV medication had been found to be more significant in HCV male patients .The relation between possible risk factor for transmission of HCV and residence, viral load were not significant.

Conclusion: The most common probable risk factors as single risk factor for the male was blood transfusion, and for female was caesarean section. While for two risk factors for male were Blood transfusion and Hemodialysis and for female were Caesarean section & Dental procedure and for both gender were hemodialysis & dental procedure.

While for three risk factors for both gender were surgery, barbers, and dental procedure. In our study we had found the prevalence of HCV in our Iraqi big sample was relatively low. The most common genotype was genotype I then genotype IV with rarity of genotype III 0 and genotype II.

Keywords: hepatitis C, genotype, prevalence, transmission

1. Introduction

HCV present in all continents, and about 170 million patients are chronically infected with HCV. In industrialized countries, the incidence of HCV infection has decreased considerably due to blood screening and measures that prevent HCV infections in intravenous drug users (Goldman & Schafer, 2015; Moradpour & Penin, 2013).

However, according to the Centers for Disease Control and Prevention about 17,000 new cases of acute HCV still occur annually in the United States. In France, about 2500 new infections occur yearly. In developing areas of the world; HCV incidence and prevalence are higher in which the main route for HCV infection is unsafe medical or surgical procedures, only about 50% of blood products are screened for Anti-HCV antibodies in these countries, and about 40% of all injections are assumed to be given with reused equipment (Moradpour & Penin, 2013; Feldman, Friedman, & Brandt, 2015).

Worldwide; three different epidemiologic patterns of HCV infection have been identified. They are (1) previous exposure through health care with peak prevalence in older patients; (2) exposure due to IV drug using; the major risk factor since about 1960. When data first became available with peak prevalence among middle-aged persons; and (3) ongoing high levels of HCV infection in areas in which high rates infection occur in all age groups (Cheifetz, Brown, Curry, & Moss, 2011).

All over the world; Subtypes {1a} and {1b} predominate. In the United States; the most common genotypes are {1a} and {1b} about75%, {2a} and {2b} about 15%, and {3a} about 7%. Genotype {3a} is more prevalent in Western Europe where it accounts approximately 35% of cases, specifically among intravenous drug users.

Genotype {4} is highly prevalent in Middle East and Africa. Its incidence and prevalence had been found to be increase in IV drug users in industrialized countries. Genotype {5} is rare outside South Africa, and genotype {6} is rare outside Southeast Asia, Genotype VII has been reported in Canada from central African immigrants (Goldman & Schafer, 2015; Cheifetz et al., 2011).

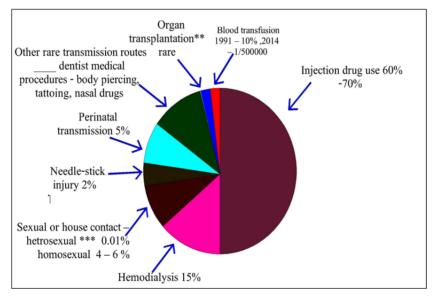


Figure 1. Common ways of transmission of hepatitis C

2. Patients and Methods

In this cross sectional retrospective study; from February 2017 to February 2019 we studied all people who attend four big hospitals, three of them in Baghdad: (Baghdad medical city, Al- Yarmouk teaching hospital, Al-Nu'man teaching Hospital) and one hospital in Basrah (Al-Basrah teaching hospital).

Those people attended hospitals before surgery or pregnant or marriage, or seeking for medical treatment for advice for their complaint, all were screen for hepatitis C, any positive result was recorded in the lab file or sent by the patient to GIT and medical consultation department where it also recorded in specific file. So all people had been documented in one or these two places, their total number were 165273 and we studied all looking healthy people and who attending main Baghdad blood bank for donation who were screen for hepatitis C, the total number was 190625 persons, so by collection of both samples from the hospital and blood bank the total number was 355898, 232645 were males, and 123253 were females, total persons who were positive for hepatitis C were 1155, males were 650 (56%) and females 505 (44%).

Further evaluation of positive patients were done looking for their viral load by PCR and genotype for hepatitis C, both of these lab tests were done in private lab because they were not available in those hospitals, positive persons

were recorded in special file and signed for that in order not to donate again and we took documentation form these files.

The questionnaire in this study for multiple risk factors for HCV transmission included;

- 1). History of blood transfusion
- 2). Hemodialysis
- 3). Caesarean section for female.
- 4). Injured male during shaving by barber.
- 5). Patients who had dental procedure.
- 6). Surgical operation.
- 7). Tattooing.
- 8). Cupping.

9). Improper use of IV medication (by intravenous route without medical prescription).

- 10). Body piercing.
- 11). Sexual or household.
- 12). Organ transplant.
- 13). Needle stick.
- 14). Perinatal.
- 15). Drug abuse (intravenous drug abuse IVDU).

We did evaluate the prevalence of the genotype and the viral load and it's relationship to the root of transmission so we consider:

- 1). High viral load (>800000 IU/ml).
- 2). Low viral load (<800000 IU/ml). (Koziel, 1997; Franciscus et al., 2011; Carey, 2003)
- 2.1 Statistical Analysis

Each patient assigned a serial identification number. The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 20. The categorical data presented as frequency and percentage tables.

The continuous data were represented by mean, standard deviation and range. The Chi-square was used to assess the association between categorical variables. P – Value less than 0.05 was used as the alpha level of significance. Finally all findings and results were presented in tables and figures with an explanatory paragraphs for each table and figure.

3. Results

Patients who had been confirmed to have hepatitis C positive (1155) patients of these, (505) patients were female and (650) patients were male. Their ages were ranging from (19) to (78) years with mean age at presentation = 48.1 (standard deviation [SD] \pm 15.8) years. Also their mean age at diagnosis = 47.3 (SD \pm 15.6) years.

BMI normal value are:

Underweight = <18.5

Normal weight = 18.5-24.9

Overweight = 25-29.9

Obesity = BMI of 30 or greater

Their body mass index (BMI) was divided into:

1) Below normal weight; <18.5 kg/m² were 9%

2) Normal weight; $18.5 - 25 \text{ kg/m}^2$ were 62%

3) Over weight; ≥25 kg/m2 were 29%. (WHO, 2014)

The mean body mass index = 22.9 (SD \pm 3) kg/m², Their BMI range (17 – 29) kg/m².

Table 1. Socio-demographic characteristics of cases with HCV infection

Variables	Number	%
Gender		
Male	650	56
Female	505	44
Residence		
Urban	914	
Rural	241	
Body mass index categories		
$< 18.5 \text{ Kg/m}^2$	96	9
$18.5 - 25 \text{ Kg/m}^2$	684	62
$>= 25 \text{ Kg/m}^2$	375	29
No. of partners		
Single	117	10
Married	1038	89.9
Occupation		
Housewife	490	52
Constructor	309	20
Retired	101	9
Student	18	4
Teacher	17	4
Healthcare worker	60	1
Others	140	10
Total	1155	100%

Mean age at presentation = 48.1 (standard deviation [SD] ± 15.8) years.

Mean Age at diagnosis = 47.3 (\pm 15.6) years, Range (19 – 78) years.

Body mass index = 22.9 (\pm 3) kg/m², Range (17 – 29) kg/m².

3.1 Prevalence of HCV in Studied Sample

Prevalence of HCV in studied sample

Point prevalence	Female	Male	All
Per 100%	0.41%	0.28%	0.32%
D 100 000 (059/ CD)	409.7	279.4	324.5
Per 100,000 (95%CI)	(406.2 - 413.3)	(277.3 – 281.5)	(322.7 – 326.4)

95%CI: 95 percent confidence interval.

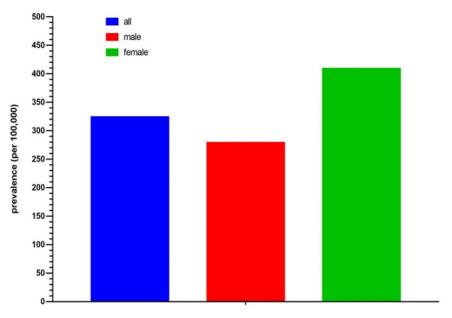


Figure 2. Prevalence of HCV

3.2 HCV Characters Among the Studied Cases

From the (1155) patients, viral load was done in (746) patients which range (5200 – 10500000 IU/ml) and their Median is 688000 IU/m. (356 patients had high viral load (>800000 IU/ml) to comprise (48%), while (390) patients had low viral load (<800000 IU/ml) and comprise (52%).

Also from the (746) patients, only (653) patients had done their genotype.

Genotype I is found in (345) patients to comprise (52.8%) genotype Ia 152 and Ib 193, and genotype 4 is found in (301) patients to comprise (46.2%), while genotype III is found only in 7 case to comprise (0.5%) and genotype II in 2 cases 0.3%. As shown in Table 2.

Table 2. Characteristics of HCV among the studied cases

Variables	Number	%
Viral load		
High viral load (>800000 IU/mL)	356	48
Low viral load (<800000 IU/mL)	390	52
Total	746	100
Median (Range) IU / mL	688000 (5200-10500	000)
Genotype		
Ι	345	52.8
Ia	152	-
Ib	193	-
IV	301	46.2
III	7	0.5
II	2	0.3
V	0	0
VI	0	0

3.3 Number and Percentage of Cases, According to Possible Risk Factors for HCV Transmission

Patients with blood transfusion history were (536) to comprise (53%), those with dental procedures surgery are (425) to comprise (42%). Those who underwent hemodialysis whom were negative for hepatitis C before starting hemodialysis and then got positive result hemodialysis their number is (364) to comprise (36%).

Female sustained Caesarean section (C.S) were (193) from total number of 505 of female to comprise (34.5%), while injured male patients during shaving by barbers are (216) from total 650 to comprise (33%).

Patients who have history of surgical operation were 243 to comprise (24%). while only (84) patients had done tattooing comprising (8%). patients with history of cupping are (67) to comprise (6%), and number of patients who took intravenous injections by their own without medical prescription (improper use of IV medication) is (45) to comprise (4%).and (43) patients had done body piercing comprising (4%).

21 patients had Sexual history (heterosexual) i.e.; 2% of total. Also 22 patients had organ(Renal) transplantation who were negative before that, and comprise (2%),while only 11 patient had needle stick injury(nurse) to comprise (1%),and no case with history of perinatal,intravenous drug abuse(IVDU) or idiopathic.

These data are presenting in Table 3:

Table 3. Number and percentage	ge of cases, according to	possible risk factors f	or HCV transmission
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Risk factor	No (1155)	%
History of blood transfusion	536	53
Dental procedure	425	42
Hemodialysis	364	36
Caesarean section (total=55)	191	34.5
Injured males by barbers (total=45)	216	33
Surgical operation	243	24
Tattooing	84	8
Cupping	67	6
Improper use of IV medication	48	4
Body piercing	46	4
Sexual or household	21	2
Organ transplant	22	2
Needle stick	11	1
Perinatal	0	0
Drug abuse	0	0
Idiopathic	0	0

*no. of females with history of caesarian section per total 505 females.

**no. of males with history of injury by barbers per total 650 males.

3.4 Average Age at Diagnosis, According to Patients 'Gender'

Male	Female	
$\mathbf{N} = 650$	N = 505	P value
Mean ± SD	Mean ± SD	
50 ± 15.7	45.1 ± 15.4	0.119
	N = 650 Mean ± SD	N = 650 N = 505 Mean ± SD Mean ± SD

Table 4. Average age at	diamosis	according to	nationts'	render
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Student's t-test, SD= Standard deviation.

3.5 Average Age at Diagnosis, According to Possible Risk Factor for Transmission

By this we found that mean age at diagnosis for patients with history of body piercing and history of organ transplantation was younger than other risk factors which their mean age at diagnosis was at middle age. As shown in Table 5:

Table 5. Average age at diagnosis, according to possible risk factors of transmission

Risk factor	Age at diagnosis
Kisk factor	Mean±SD (Range)
Improper use of IV medication	60.75±12.74 (44-75)
History of blood transfusion	50.87±15.13 (19-75)
Hemodialysis	48.58±14.98 (20-75)
Sexual or household	52.5±17.68 (40-65)
Surgical operation	55.12±14.52 (27-78)
Body piercing	25.75±4.57 (20-31)
Needle stick	41
Organ transplant	27.5±10.6 (20-35)
Cupping	54.7±9 (44-64)
Tattooing	48.1±16.4 (31-75)
Female C/S	42±14.6 (22-75)
Shaving among males	48.6±11.8 (23-62)
Dental procedure	48.9±15 (20-75)

3.6 Relation Between Number of Possible Risk Factors for HCV Transmission and Number of Patients for That Number

By this relation we find that there are (398) patients have history of single risk factor of transmission, (380) patients have history of two risk factors of transmission, (248) patients have history of three risk factors of transmission while (124) patients have history of four risk factors of transmission.

For the single risk factor, (178) patients have history of blood transfusion due to anemia, peptic ulcer, menorrhagia, pregnancy and hemorrhoids .Also we have (56) patients for dental procedure and 52 for caesarian section(c/s), (38) for surgical operation and injured male by barbers during shaving was 35while (13) patient for each hemodialysis, body piercing was 14 and tattooing was 13 as shown below in Table 6.

Table 6. Number of possible risk factors of transmission and patients Number

Risk factors	Number of patients
Single risk factor	N = 398
Hemodialysis	13
Piercing	14
Tattooing	18
barbers	35
Surgery	37
Dental procedure	56
Caesarean section	252
Blood transfusion	178
Two risk factors	N = 380
Hemodialysis, Cupping	17
barbers, Dental procedure	16
Blood transfusion, Cupping	17
Cupping, Dental procedure	18
Piercing, Dental procedure	19
Cupping, Tattooing	14
Hemodialysis, Transplant	13
Blood transfusion, Dental procedure	15
Hemodialysis, Caesarean section	25
Blood transfusion, Caesarean section	23
Surgery, Dental procedure	24
Blood transfusion, Surgery	33
Hemodialysis, Dental procedure	41
Caesarean section, Dental procedure	47
Blood transfusion, Hemodialysis	58
Three risk factors	N = 248
Hemodialysis, Surgery, barbers	16
Blood transfusion, Hemodialysis, Caesarean section	17
Blood transfusion, Hemodialysis, Surgery	15
Hemodialysis, Sex household, Dental procedure	12
Blood transfusion, Hemodialysis, barbers	13
Blood transfusion, Hemodialysis, Caesarean section	11
Blood transfusion, Hemodialysis, Transplant	14
Blood transfusion, Surgery, barbers	13
Blood transfusion, Caesarean section, Dental procedure	12
Cupping, Caesarean section, Dental procedure	13
Blood transfusion, Surgery, Dental procedure	13
Sex household, barbers, Dental procedure	12
Blood transfusion, Hemodialysis, Surgery	22
Blood transfusion, Hemodialysis, Dental procedure	31

Surgery, barbers, Dental procedure	32
Four risk factors	
Improper use of IV medication, Hemodialysis, Surgery,	16
Dentists	
Blood transfusion, Hemodialysis, Needle stick, Tattooing	17
Blood transfusion, Hemodialysis, Caesarean section, Dentists	18
Hemodialysis, Tattooing, barbers, Dentists	12
Blood transfusion, Surgery, Tattooing, Caesarean section	11

3.7 Relation of Cases' Gender With Possible Risk Factors of Transmission

	Gender				
Risk factor	Male		Female		p-value
	No. (N=650)	%	No. (N=505)	%	
History of blood transfusion	232	51.1	300	54.5	0.732
Dental procedure	194	42.2	23	41.8	0.968
Hemodialysis	211	46.7	157	27.3	0.044
Surgical operation	132	28.9%	119	20.0%	0.3
Tattooing	24	4.4%	67	10.9%	0.236
Cupping	41	8.9%	23	3.6%	0.271
Improper use of IV medication	41	8.9%	0	0%	0.024*
Body piercing	0	0%	40	7.3%	0.065
Sexual or household	22	4.4%	0	0%	0.114
Organ transplant	21	4.4%	0	0%	0.114
Needle stick	0	0.0	19	1.8	0.363

Table 7. Relation of cases' gender with possible risk factors of transmission

Chi-square test, * Significant at 0.05 level.

3.8 Relation of Cases' Residence With Possible Risk Factors of Transmission

According to the residence wither urban or rural areas, there are (90) patients from urban area and (10) patients from rural area .The risk factors with high percentages in urban area are dental procedure, hemodialysis, surgical operation, cupping, sexual contact, organ transplantation and needle stick injury.

While in rural area the risk factors of high percentages are blood transfusion, tattooing, improper use of IV medication and body piercing.

Significant p-value have not been found as shown in Table 8.

Risk factor	Residence				
	Urban		Rural		p-value
	No. (N=1029)	%	No. (N=126)	%	_
History of blood transfusion	380	42	63	50	0.640
Dental procedure	340	33	40	31.7	0.892
Hemodialysis	204	24%	28	22%	0.267
Surgical operation	122	12%	29	22%	0.755
Tattooing	70	7%	11	0.8%	0.806
Cupping	63	6%	0	0%	0.4
Improper use of IV medication	30	3%	11	0.8%	0.306
Body piercing	30	2%	9	0.7%	0.306
Sexual or household	22	2%	0	0%	0.634
Organ transplant	21	2%	0	0%	0.634
Needle stick	19	1.2	0	0.0	0.738

Table 8. Relation of cases' residence with possible risk factors of transmission

Chi-square test.

3.9 Relation of Viral Load Categories With Possible Risk Factor for Transmission

From the total (1155) patients; only (746) patients had done their viral load. From the (350) patients; (390) patients with low viral load (<800000 IU/ml), and (350) patients with High viral load (>800000 IU/ml).

For patients who have history of tattooing, body piercing, sexual contact, organ transplantation and needle stick injury have been found to have high percentage of low viral load compared with other patients who have same risk factor but with high viral load.

For patients who have history of blood transfusion, hemodialysis, dental procedure, surgical operation, cupping and improper use of IV medication have been found to have high percentage of high viral load compared with other patients who have same risk factor but with low viral load.

P-value regarding this relation was not significant. As shown in Table 9.

Risk factor	Low viral load		High viral load		
	No. (N=390)	%	No. (N=356)	%	— p-value
History of blood transfusion	182	46.0	181	50.0	0.469
Dental procedure	148	37.0	175	49.0	0.263
Hemodialysis	143	36%	132	37%	0.912
Surgical operation	72	18%	98	25%	0.278
Tattooing	28	7%	22	6%	0.735
Cupping	29	7.4%	4	2.5%	0.358
Improper use of IV medication	17	4.3%	24	8%	0.493
Body piercing	0	0%	28	8%	0.13
Sexual or household	22	5.6%	0	0%	0.174
Organ transplant	21	5%	0	0%	0.174
Needle stick	12	3.0	0	0.0	0.34

Table 9. Relation of viral load categories with possible risk factor for transmission

Chi-square test.

4. Discussion

In our study we had found the prevalence of HCV in our Iraqi big sample was 0.41% in female, 0.28% in male and 0.32% in all, and most common genotype was genotype I 52.8% then genotype IV 46.2% with rarity of genotype III 0.5% and genotype II 0.2%, while the most common occupation for HCV patients is housewife (52%) and constructor (20%) which resemble other study done in Egypt at 2014 by Adinolfi et al. (2001).

while health care worker (nurse) comprise (1%) only. while in the world the occupation which consider a risk factor for HCV acquisition is health care worker where significant prevalence had been found in USA, North Africa, Middle East and South Asia with low prevalence in Europe and no increase prevalence in Japan by study done by Claudia Westermann et al. at 2015 (Westermann et al., 2015).

By our study; the more frequent genotype is 1 then 4. while the genotype 4 is the more frequent in Arabian peninsula region (Saudi Arabia, Yemen, Oman, Bahrain, Qatar, UAE, Kuwait), Sham region (Iraq, Syria, Lebanon, Gaza Strip except Jordan), also in Egypt, Sudan and Libya. While genotype 1 is the more frequent in Jordan and Morocco by study done at 2012 by Mohamed A. Daw et al which mention the more frequent genotype in Arabian world (Daw & Dau, 2012).

While in a study done by Farahnaz Fallahian et al at 2011; the genotype 1 is the more frequent in Iraq, Bahrain, Jordan, Iran and Turkey.

While genotype 4 was more frequent in Saudi Arabia, Syria, Lebanon, Egypt and Sudan, while genotype 3 was the more in Pakistan.

In Europe, the more prevalent genotype is 1 followed by 3 and 2 (European Association for Study of Liver, 2014). While In the United States; genotype 1 is the more prevalent of HCV infections followed by genotype 2 then 3 while genotype 4, 5, or 6 is the less frequent types (Blatt et al., 2000).

In retrospective study had done by Ekram ABD EL-WAHAB et al in Egypt at 2014 for factors associated with HCV transmission (El-Wahab, Mikheal, Sidkey, & Shatat, 2014), the results were as follow(percentages of patients reported history of risk factor for transmission); Barbers(sharing manicure set) 72.7%, Parenteral treatment range from once time 14.4% to more than once 72.2%, Body piercing 52.8% while blood transfusion 51%, Injection anti bilharzial drug 32.3%, Tattooing 30.8% while cupping 28.3%, Surgical operation range from minor 16.9% to major 30.1%.

By comparism these results to our study; there are somewhat similarity with blood transfusion, dentists, surgical operation and needle stick injury. While tattooing, cupping, body piercing, drug abuse and barbers are higher than our percentages results.

By study done at 2005 in Turkey by Beytullah Yildirim et al; the main risk factors for HCV infection in Turkish community are surgical operation, multipartner sex, frequent dental therapy, dental extraction and blood transfusion (Yildirim et al., 2005), which favour our study that blood transfusion, dentists and surgical operation are from the main routes of HCV transmission (Yildirim et al., 2005).

In the developed world (United States, Russia and China); the primary risk factor for HCV transmission is intravenous drug use (Nelson et al., 2011).

In the united states the percentages of risk factors for HCV transmission are : Injection drug use 60%, sexual contact 15%, blood transfusion (before screening) 10%, unknown 10% and other (health care work, hemodialysis, Perinatal) 5% (Nelson et al., 2011; Xia, Luo, Bai, & Yu, 2008).

As we notice, there are different percentages for risk factor for HCV transmission in comparism between our study and other studies in different regions in the world and this probably due to different in social, cultural, behavioral and environmental factors.

By our study; there is relation between male gender and infection with HCV by hemodialysis and this similar to other studies had done in united states (Kalautar-Zadeh et al., 2007; Charles et al., 2005) and Europe (Italy) (by Natov et al., 2005) while there was no relation between gender and infection with HCV by hemodialysis by study done in Iraq at 2010 (Al-Rubiaei, 2010).

By our study there are no relations between risk factor for HCV transmission and patients residence, while by other studies done in Egypt (by Mohamed et al., 1996; Guerra et al., 2012) and Australia (Roberts & Algert, 2000) had shown that HCV infection increase in rural areas (Pallás et al., 1999).

5. Conclusions

By our study we found there are multiple risk factor for infection by HCV in Iraqi patients, and the most probable

common risk factors are hemodialysis, dental extraction, surgical operation, blood transfusion, caesarean section, injured male by barbers and. Most probable risk factor for patients with HCV infection who had history of single route of transmission is blood transfusion. While the most common two result was dental procedure and caesarean section.

Also there are significant transmission of HCV by hemodialysis and improper use of IV medication with male patient.

There are difference in prevalence of risk factors for HCV transmission in HCV Iraqi patients and other regions in the world which belong to differences in social, cultural, behavioral and environmental factors.

The incidence of genotype I is 52% is more than genotype IV is 465 in our study. While genotype III was 0.5% and II only 0.3%.

-from total 355899 persons; 1155 were positive for HCV (0.32), male = 232645 and 650 of them were positive (0.28) and female = 123253 and 505 of them were positive (0.41).

Competing Interests Statement

The authors declare that there are no competing or potential conflicts of interest.

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