Association of Hepatitis C viral load with liver functions and risk factors among HCV patients, Minia governorate, Egypt

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Abstract

Hepatitis C virus (HCV) is one of the blood transmitted hepatitis viruses. HCV infections have been identified as major causes of chronic hepatic diseases, and hepatocellular carcinoma. The aims of the current study were to determine the HCV viral load between 35 hepatitis C patients in Minia governorate, Egypt, and to assess association of the viral load with abnormal liver functions including; Alanine aminotransferase (ALT), prothrombin activity and platelet count. In addition to assessing if there are any risk factors associated with the population group, sex, age and other factors. About 35 blood samples were collected from hepatitis C patients randomly selected from the outpatient clinic at the Viral Hepatitis Management Center, Minia governorate, Egypt; including males and females of different ages. Viral load was determined using Real-time polymerase chain reaction (RT-PCR). All relevant information was collected from each patient including personal and clinical data. Current results showed that 68.60 % of the samples were from males and 31.4 % were from females, and most of them aged between 51 and 70 years. Approximately 11 (31.4 %) of the HCV patients had viral loads of <106, 12 (34.3 %) recorded viral loads of <105, and about 12 cases (34.3 %) had a viral load of < 104. HCV infection has been associated with 4 risk factors representing high HCV transmission routes including; dental intervention (80.0 %), history of hospital admission (65.7 %), previous surgeries (57.1 %) and family history of HCV (48.6 %). However, history of Schistosomiasis and blood-transfusion showed low association with HCV infection; recording (31.4 %) and (22.9 %), respectively.

Keywords: Hepatitis, HCV, Liver functions, RT-PCR, Risk factors

1. Introduction

Hepatitis C virus (HCV) is a viral pandemic, which represents the most common blood-borne viral infection that preferentially replicate in the liver (Karoney and Siika, 2013; Tabata *et al*., 2020).

at -20°C for further investigations, in reference to Halfon *et al*., (1996).

2.2. Extraction of HCV-RNA

Viral RNA was extracted from patients' sera using QIAamp DSP virus Kit (cat#60704, Qiagen, Germany) according to the manufacturer's instructions, and the eluted RNA was stored at -80°C until further use in HCV-RNA quantification.

According to the method conducted by Taha *et al*., (2017), there were 4 stages during the extraction procedure mainly; lysis, wrapping, cleaning, and elution. A proteinase K and a lysis buffer have been used, which ensure inactivation of the RNase enzyme and digestion of the viral coat proteins. The lysed samples were applied to the binding buffers and binding conditions were adjusted. To allow optimum adsorption of the viral nucleic acids to the silica-based membrane of the spin column, lysates were thoroughly mixed with magnetic particles (carrier of RNA). The viral nucleic acids remained attached to the magnetic particles during a series of washing steps using first wash buffer 1, wash buffer 2 and then ethanol, while the contaminants were effectively washed away. Highly purified viral nucleic acids were eluted in the adsorbed viral nucleic acid elution buffer (AVE).

2.3. Quantification of the HCV-RNA

The HCV-RNA was amplified on Stratagene Mx3000 PTM using Artus HCV QS-RGQ-PCR Kit reagents. Amplification followed by simultaneous detection was carried out using RT-PCR, with TaqMan assay for a particular region of the HCV genome.

The reaction mixture for RT-PCR was prepared in a single eppendorf tube containing all components needed for PCR assay including; buffers, reverse transcriptase, and Taq Polymerase enzyme. The amplification reaction was carried out as follows; 6 µl of HCV RG Master A, 9 µl f HCV RG Master B and 10 µl of the extracted RNA of a serum sample were added to complete the reaction final volume to 25 µl, in reference to Elsawaf *et al*., (2015).

The reaction occurred under the following temperature conditions; incubation for 30 min. at 50°C, to reverse transcription (RT) of HCV viral RNA, to cDNA, followed by AmpliTaq gold activation for 15 min. at 95°C, 45 cycles of three PCR amplification steps, denaturation for 30 sec at 95°C, annealing for 1 min. at 50°C, extension for 30 sec at 72°C, and finally fluorescence detection.

The HCV probe is a small linear oligonucleotide that is labeled with a 5′-end fluorophore and a 3′-end quencher. The target of the PCR primers and probe is the conserved 5′ un-translated region of the HCV genome. The HCV probe hybridizes preferentially to the HCV target sequence, which allows fluorescence emission and detection (Halfon *et al*., 2006).

2.4. Statistical analysis

Sets of patient's data were analyzed using Microsoft® Excel software to produce charts and tables shown in the section of results. Moreover, data statistical analysis was done using the SPSS version 16. Qualitative variables were described as numbers and percentages. T-test of independent samples was used for parametric quantitative data, Mann Whitney test for non-parametric quantitative data, while Fisher's exact test was used for comparison between groups as appropriate. Significant level at P value < 0.05.

3. Results

3.1. Extraction and quantification of HCV-RNA by real time RT-PCR

Real time RT-PCR amplification curves of HCV-RNA are shown in Fig. (1). Values for HCV- RNA are reported in IU/ml. Of our 35-HCV patients 12 (34.3%) have viral loads of < 104, 12 patients (34.3%) their viral loads are of <105, and 11 cases (31.4%) have a viral load of < 106 (Table 1, Fig. 2).
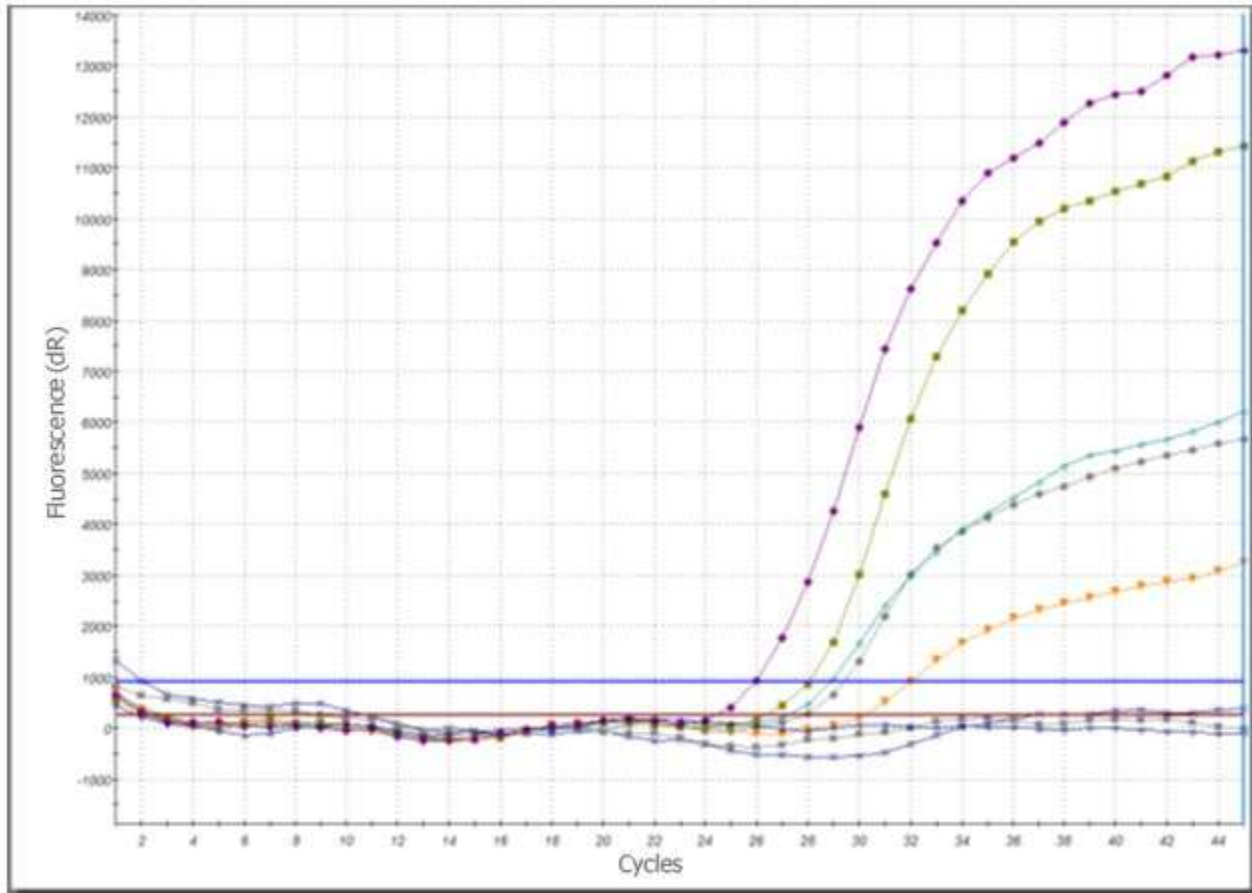
Fig. 1: Real time amplification curves of HCV-RNA by real time RT-PCR assay. The figure shows amplification plots of HCV cases showing positive cases with different threshold cycles (Cts)

Table 1: Viral load of the 35 HCV patients

Viral load (IU/ml)	No.	%
$> 10^3 - <10^4$	12	34.3
$> 10^4 - < 10^5$	12	34.3
$> 10^5 - <10^6$	11	31.4
Total	35	100
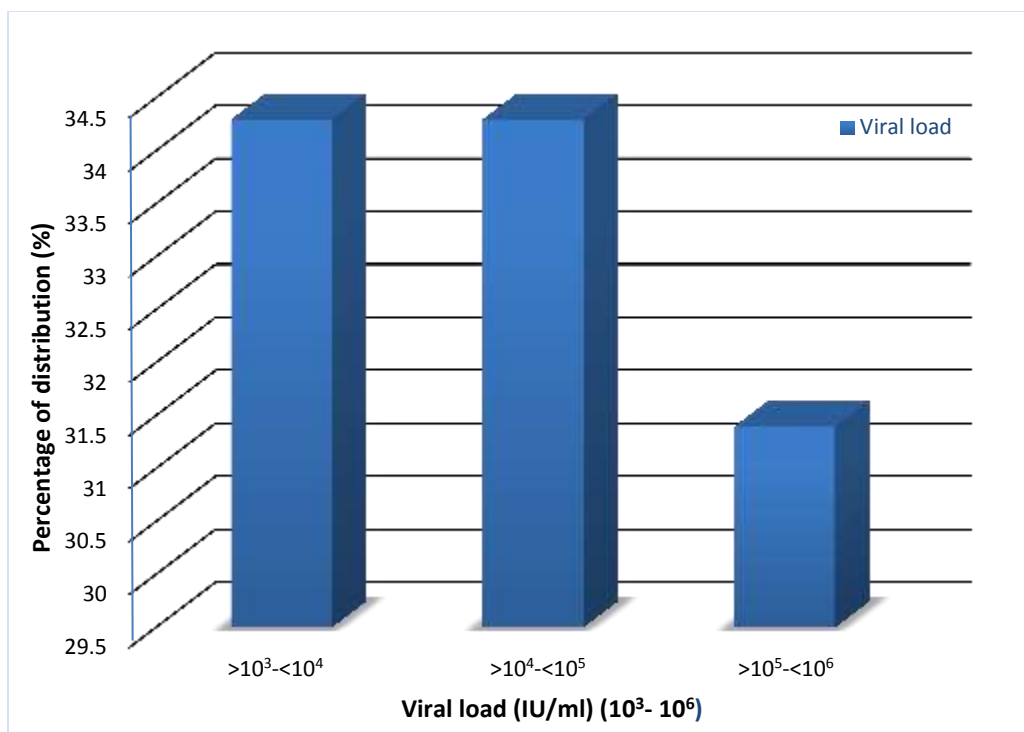
Fig. 2: Distribution of the 35 studied patient's samples viral load

3.2. Data analysis

Ages of patients involved in the study ranged from 23-75 years old. Out of these samples, 24 (68.60 %) were males, and 11 (31.40 %) were females. About 60.0 % of the patients aged 51-70 years, 4 (11.4%) patients were present in each age group of 20-30, 31-40 and 71-80, while those in the age group 41-50 years were 5.7% (Table 2).

The highest prevalence of HCV 9 (25.7 %) is in males of age groups of 51-60 years, followed by 61-70 years age-groups with prevalence of 6 (17.1 %); however, age groups from 31-40 and 71-80 years showed HCV prevalence of 3 (8.6 %) for each.

Age-groups from 20-30 and 41-50 years showed lowest prevalence of 5.7% and 2.9%, respectively. On the other hand, females predominant cases are in age groups of 51-60 years with 5 (14.2 %), followed by age groups of 20-30 and 41-50 years with 2 (5.7 %)

prevalence in each. The prevalence of age groups from 31-40 and 61-70 years is 1 (2.9 %) in each, whereas no female cases (0.0 %) are recorded in the 71-80 years age group, as demonstrated in Fig. (2, 3).

Out of all the HCV patients, about 25 (71.4%), 27 (77.1 %) and 28 (80.0 %) cases had normal ALT, platelet count and prothrombin levels, respectively (Fig. 4). The viral load is not significantly associated with the elevated ALT, platelet count and prothrombin abnormalities, recording; $P = 0.152$, $P = 0.154$ and $P = 0.087$, respectively (Table 3).

Many health care risk factors are associated with HCV infection showing different distribution among the current 35 HCV patients (Fig. 5). These risk factors include; history of dental intervention 28 (80.0 %), history of hospital admission 23 (65.7%), surgery 20 (57.1 %), family history of HCV infection 17 (48.6 %), history of schistosomiasis and blood transfusion 11 (31.4 %), 8 (22.9 %), respectively (Table 4).

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Table 2: Distribution of the 35 HCV patients according to their ages and gender

Age	Male		Female		Total	
	No	%	No	%	No	%
20-30	2	5.7	2	5.7	4	11.4
31-40	3	8.6	1	2.9	4	11.4
41-50	1	2.9	2	5.7	3	5.7
51-60	9	25.7	5	14.2	14	40
61-70	6	17.1	1	2.9	7	20
71-80	3	8.6	0	-	3	11.4
Total	24	68.6	11	31.4	35	100
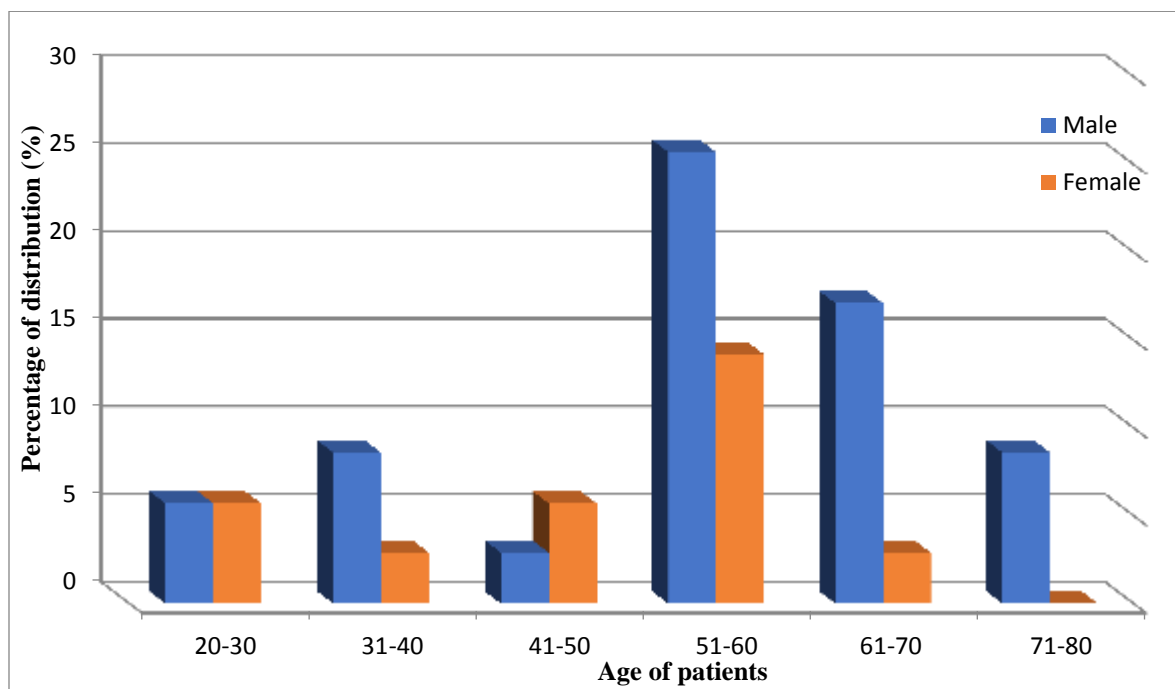
Fig. 3: Distribution of the 35 HCV patients according to their age and sex

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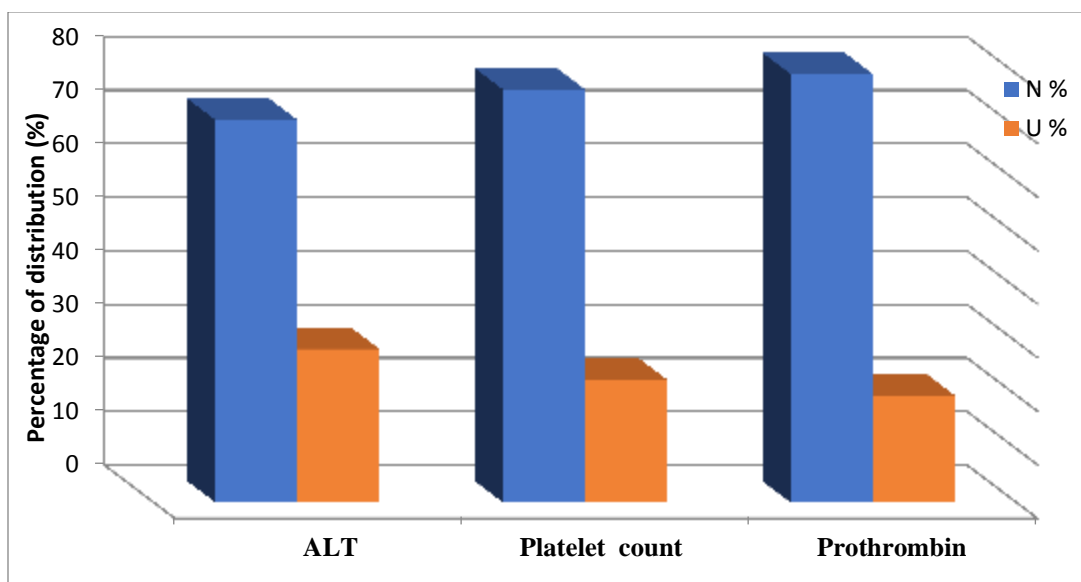
Fig. 4: The ALT, Platelet count and Prothrombin activity among the 35 HCV patient's cases. Where; N: Normal, U: Up-normal

Table 3: HCV viral loads correlations with liver functions abnormalities

	Viral load	
	r	P value
ALT level abnormality [(S)]	0.247	0.152
Platelets count abnormality [(S)]	0.246	0.154
Prothrombin activity abnormality [(S)]	0.294	0.087

Where; (S) Spearman's correlation; r: correlation coefficient, significant level at P value < 0.05

Table 4: Distribution of risk factors among the 35 HCV patients

Risk factors	No	%
Dentistry	28	80.0
Hospital admission	23	65.7
Surgery	20	57.1
Family History of HCV	17	48.6
Anti-schistosomal treatment	11	31.4
Blood Transfusion	8	22.9

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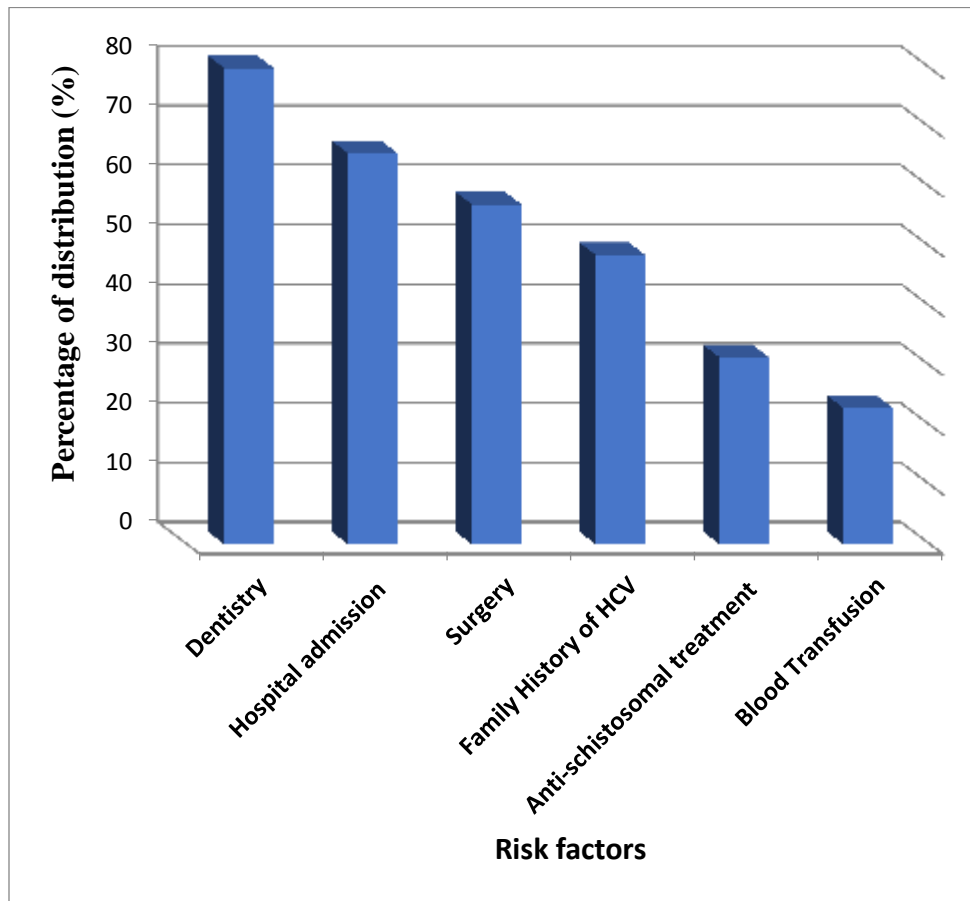
Fig. 5: Distribution of risk Factors among the 35 HCV patients

For community-based dental interventions, the strength of correlations is 19 out of 24 (5.72 %) male cases, compared to 9 out of 11 (3.45 %) female cases, with non-statistical significance (P = 1). The admission to hospital is related to 16 HCV male status (69.6 %) compared to 7 females (30.4 %) with non-statistical relevance (P=1). Undergo to previous surgery associate in 13 (65 %) males and 7 (35 %) females of current HCV patients, showing non-statistical significance (0.721). History of HCV infections among families and history of anti-shistosomiasis each show more distribution between males than females, presenting non-statistical significance (P = 0.725), (P = 1), respectively. In those patients who have undergone blood transfusion (5 patients), HCV is less common than in those who have not received blood (30 patients), demonstrating non-statistical significance in male to female distribution (P = 0.64). Different reported risk factors associated with HCV infections show non-statistical significance correlation with the ages and viral loads, as shown in Table (5).

Table 5: Association of HCV infection with different risk factors among 35 HCV cases

Risk factors		Sex		Age	Viral load ($\times 10^3$)
		Male	**female**		
Dentistry	Yes	19(67.9%)	9(32.1%)	53±14	201/(93-1075)
	No	5(71.4%)	2(28.6%)	53±17	93/(90-1030)
P value		1		0.922	0.523
Hospital	Yes	16(69.6%)	7(30.4%)	55±14	520/(107-1200)
Admission	No	8(66.7%)	4(33.3%)	50±16	96/(83-420)
P value		1		0.378	0.095
Surgery	Yes	13(65%)	7(35%)	51±15	257/(92-1075)
	No	11(73.3%)	4(26.7%)	56±13	112/(75-1030)
P value		0.721		0.284	0.629
Family history	Yes	11(64.7%)	6(35.3%)	52±15	182/(93-550)
of HCV	No	13(72.2%)	5(27.8%)	54±15	235/(79-1100)
P value		0.725		0.696	0.934
Anti-schistosomal	Yes	8(72.7%)	3(27.3%)	53±15	545/(98-1100)
Treatment	No	16(66.7%)	8(33.3%)	53±14	144/(81-807)
P value		1		0.948	0.268
Blood	Yes	3(60%)	2(40%)	60±9	550/(182-563)
Transfusion	No	21(70%)	9(30%)	52±15	111/(87-1100)
P value		0.64		0.257	0.409

Where; Independent Samples (T) test for parametric quantitative data (expressed by mean) between the two groups; Mann Whitney test for non-parametric quantitative data (expressed by median) between the two groups; Fisher's exact test for qualitative data between the two groups. -Significant level at P value < 0.05

4. Discussion

This study was performed in Minia governorate of Egypt, where the majority of population lives in rural areas. Seropositive cases in the current study remained higher in 51-60 years (38.9 %), followed by 61-70 years (22.2 %). Several other studies have also demonstrated high sero-prevalence of anti-HCV antibody among adult populations. In a recent study conducted by Vilas *et al*., (2018), the highest percentage of positive HCV was in the age group 31- 40 years. A study in India conducted by Verma *et al*., (2014) reported that adults of 21-60 years had a maximum (70 %) incidence of HCV. Moreover, Bharadwaj *et al*., (2014) revealed that the highest HCV prevalence was detected among people of 41-60 years age group. The prevalence of HCV among high age individuals suggests that they may substantially contribute to the ongoing HCV transmission in Egypt (Breban *et al*., 2014).

In this study, HCV sero-prevalence among males was higher (77.8 %) compared to females (22.2 %). Current results are in agreement with the study of El-Zanaty, (2016), who estimated that the prevalence of HCV among males are more than females, and to the findings of Bharadwaj *et al*., (2014) who also reported higher prevalence of HCV in males (0.7 %), compared to females (0.66 %). This male preponderance could be attributed to their high level of exposure to different risk factors causing transmission of HCV, due to male lifestyle.

Results of this study which demonstrated higher HCV prevalence in males than females are similar to the recent study of El-Adly *et al*., (2020), who also reported that among the tested samples, prevalence of 78.4 % were in males and 21.6 % were in females. Similarly, Niya *et al*., (2017) also recorded that out of 70 HCV patients; 54 were males and 16 were females, as statistical analysis did not record any meaningful link between age, sex and genotype variables.

On the contrary, current results are not compatible with other studies in which the prevalence of anti-HCV antibodies among females was higher than among males (Ramarokoto *et al*., 2008; Ayele and Gebre-Selassie, 2013; Abo-Amer *et al*., 2018). This study demonstrated that dental intervention is strongly associated with infection with HCV. Arafa *et al*., (2005) highlighted that in Egypt, periodontal therapy has also been reported as a possible source of HCV transmission. Also, detection of HCV-RNA on dental equipment used for treatment of HCV infected patients was reported by Piazza *et al*., (1995). Thus, strict guidelines for cleaning and sterilizing instruments between patients should be applied to decrease the risk of HCV transmission. Similarly, several other reports of Kalil *et al*., (2010); Barakat and El-Bashir, (2011) have shown the continuing occurrence of HCV in dental and medical facilities.

In our findings other routes of infection including previous hospital admission and surgeries have been recorded; 65.7 % and 57.1 %, respectively. Current results are higher than those reported earlier by Omran *et al*., (2009), who stated that previous surgeries as a possible route of infection by HCV recorded 108 (36 %). In this study, blood transfusion and history of schistosomiasis showed a low HCV association recording; 22.9 % and 31.4 %, respectively. These results agreed the recent results of El-Adly *et al*., (2020), who detected low prevalence of HCV among patients with blood transfusion and anti-schistosomal history. Similar results obtained by Omran *et al*., (2009) reported that 114 (38 %) of the 300 HCV studied patients had a history of schistosomiasis infection.

Several reports of El-Gohary *et al*., (1999); Omran *et al*., (2009) have documented the role of blood transfusion as a risk factor for transmission of HCV infection, in spite of screening for anti-HCV antibodies in the blood donors. Although several

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earlier studies reported that, in developed countries such as the USA and Europe, the prevalence of HCV antibodies in blood donors was relatively low <1 %, while in developing countries such as Nigeria, Central African countries and Egypt; the prevalence of these antibodies ranged from 6 to 28% (Tanaka *et al*., 1992; Kowo *et al*., 1995; Halim and Ajayi, 2000). Nowadays, the high sensitivity and specificity of methods used to detect HCV antibodies in blood donors decreases the chance of considering blood transfusion as a risk factor. The 35 HCV patients included in this study have a viral load ranging between >103 - <106. Several studies conducted by McGuinness *et al*., (1996); De Moliner *et al*., (1998); Araujo *et al*., (2002) have argued that there is no correlation between serum levels of HCV-RNA and the serious liver disorders. Araujo *et al*., (2002) revealed that there was no correlation between hepatitis C viral (HCV) load and the histological abnormalities of the disease. In addition, Anand and Velez, (2004) highlighted that there is little correlation between HCV viral loads and the severity or progression of the liver disease. On the contrary, Adinolfi *et al*., (2001) reported that liver damage is correlated with the HCV-RNA levels. However, a previous study conducted by Kato *et al*., (1993) indicated that HCV-RNA titers were significantly higher in patients with chronic hepatitis and cirrhosis, compared to those with milder histological changes. In the present study, viral load is not correlated significantly with abnormal liver functions including; ALT, prothrombin activity and Platelet count. According to Agrawal *et al*., (2016), the severity of HCV infection can't be predicted solely by liver function tests, and normal results do not exclude the risk of a liver disease.

Conclusion

The current study provides information on hepatitis C viral load association with liver functions and risk factors among HCV patients in Minia governorate, Egypt. The HCV sero-prevalence found to be higher among males (68.6 %) as compared to females (31.4 %). Prevalence increased with age,

and there was a sharp increase among 51-70 years age group. Abnormal liver functions including ALT, prothrombin activity and Platelet count were not significantly associated with the viral load. HCV infections showed various associations with different risk factors representing high HCV transmission routes. The dental intervention showed strong association with HCV infection (80.0 %). However, history of Schistosomiasis and blood-transfusion presented low associations with HCV infection; recording (31.4 %) and (22.9 %), respectively. The severity of HCV infection can't be predicted solely by liver function tests, and normal results do not exclude the risk of a liver disease.

Conflict of interest

No conflict of interests exists between the authors of this study.

Ethical approval

This research was carried out in compliance with the ethical principles. The approval of Faculty of Pharmacy, Minia University Ethical Committee was provided before starting this study (reference number 65/2019). The patient's consents and statement of protection of the patient's privacy are provided.

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