Study on Prevalence of Parasitic Infections among Hepatitis C Virus Patients in Egypt

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ABSTRACT

Hepatitis C virus (HCV) is a viral infection affecting 71 million people globally. The highest prevalence was estimated in parasitic infections, such as *Schistosoma mansoni, Fasciola* sp., and *Toxoplasma gondii,* which can also contribute to liver disease progression. This study aimed to investigate the prevalence of co-parasitic infections with HCV in Egyptian populations and the resulting biochemical changes in liver and kidney biomarkers. Three hundred and thirty-seven blood samples were screened molecularly for HCV and immunologically for parasitic infections using PCR and ELISA assays, respectively. The liver functions were monitored by measuring the serum glutamic oxaloacetic transaminase (GOT), glutamate pyruvate alanine aminotransferase (GPT), gamma-glutamyl transferase (GGT), total protein (TP), albumin (Alb), total bilirubin (T Bil), and alkaline phosphatase (ALK). The kidney functions were evaluated by estimation of the creatinine, uric acid, urea, sodium (Na), and potassium (K) levels. The patients were categorized

according to gender and age < 21, 21 - 50, and > 50 years. Results indicated that 120 out of 287 HCV-infected cases (41.8%) have *Schistosoma* infection, of which 57, 31, 24, and 8 cases were mono-infected and co-infected *with Fasciola*, *Toxoplasma*, and *Fasciola*/*Toxoplasma*, respectively. Ninety-nine out of 287 HCV patients (34.5%) have *F. hepatica* infection, of which 51 and 9 cases were mono-infected and *Toxoplasma* co-infected, respectively. 87 out of 287 HCV samples (30.3%) have *T. gondii* infection, of which 46 cases were mono-infected. Besides, the percentage of males in the patient groups having monoparasitic infection was between 78.2% (*Toxoplasma*) and 84.3% (*S. mansoni* or *F. hepatica*), on the other hand, the highest incidences of single infections among males (*Fasciola* and *Toxoplasma*) were over the age of 50 years, at 43.1% and 39.1%, respectively. In male patients mono-infected with *S. mansoni* (42.1%), the prevalence was in age group of 21-50 years. It was found that liver enzyme levels (GPT, GOT, Alk, and GGT) besides, kidney parameters (creatinine and urea) were more affected by the type (mono or mixed) or species of parasitic infections in HCV patients. Additionally, most of the serological parameters were significantly elevated with viral/parasitic infections, especially, in patients with high viral loads.

Keywords: Hepatitis C, *Schistosoma, Fasciola, Toxoplasma,* liver and kidney functions

1. INTRODUCTION

Hepatitis C (HCV) is a viral infection that is life-threatening in several developing countries. The World Health Organization (WHO) estimated that 71 million people worldwide are chronically infected with HCV, and that HCV-related liver disease costs over 350,000 lives each year **(1)**. Egypt has the highest HCV prevalence in the world; a 2018 meta-analysis showed that the country's antibody prevalence was 11.9% **(2)**. Chronic HCV infection generally progresses slowly, with limited advanced liver disease in the initial 10 –15 years of infection. The three major reasons for mortality in patients with HCV infection are related to liver diseases such as cirrhosis and hepatocellular carcinoma, co-infection with Human Immunodeficiency Virus (HIV), and drug overdose **(3)**. Parasitic infections coexisting with HCV may be considered another risk factor for the progression of liver diseases. *Schistosoma mansoni* infection is the main causative agent of granulomatous reactions in the liver that results in splenomegaly, portal hypertension, and hepatomegaly **(4).** Also, it has been found that both acute and chronic types of toxoplasmosis

involve the liver **(5)**. The disease is caused by *Toxoplasma gondii,* which is a widespread protozoal infection distributed all over the world. Its diagnosis relies on serological examinations because the clinical manifestations interfere with many other diseases, and microscopic examination of the parasite from patients is usually difficult. In the past three decades, human fascioliasis has raised concerns about public health, directing concern of the WHO to designate it as a neglected tropical disease (**6).** Apart from several indications, infection has also been associated with liver fibrosis in both people and animals (**7**). Following *Fasciola* infection, one can develop acute cholecystitis, biliary blockage, and liver abscesses, which frequently require abdominal surgeries (**8)**. The co-occurrences and associated morbidities of parasitic diseases with HCV infection have directed our attention to studying the prevalence and risk factors as well as related liver and kidney morbidities among study participants. Therefore, the present study aimed to investigate the prevalence of co-parasitic infections with HCV and associated serum biochemical changes in terms of liver and kidney functions.

2. MATERIALS AND METHODS

2.1.Demographic features of the tested samples

A total number of HBV free 337 samples were screened for HCV with or without parasitic infections. As described in **Table (1),** the tested samples were categorized according to gender and age. The number of male samples was 267 out of 337, and the number of females was 70, where the ratio between them was nearly 4:1. The age distribution of the screened individuals was: 30 individuals (8.9%) had an age < 21 years, while 132 (39.16%) were between 21 and 50 years, and finally, 175 individuals (51.9%) were >50 years old.

Table 1: Shows the demographic characteristics of the tested patients

Sample No.	**Sex**		
No. / Age (year)	**Male (%)**	**Female (%)**	**Total (% to study)**
< 21	23	7	30
	(6.82%)	(2%)	(8.90%)
21 - 50	106	26	132
	(31.45%)	(7.7%)	(39.16%)

> 50	138 (40.94%)	37 (10.97%)	175 (51.92%)
Total	267	70	**337**
(%)	79.2	21.3	

2.2.Collection of Blood Samples and the viral detection

A total of 350 blood samples were collected from patients (The laboratories of the Armed Forces for Medical Research, AFLMR). Clear sera were separated from the collected blood by centrifugation at 3000 rpm. HCV was molecularly detected using QIAsymphony assay (integrating automated polymerase chain reaction PCR assay) with (PCR) kit (QIAsymphony DSP Virus Kit, QIAGENCAPANY, Germany). The HCV positive sera samples were immunologically retested to HBV infection by enzyme-linked immunosorbent assay (ELISA) using VITROS® 3600 Immunodiagnostic System, QuidelOrtho™, USA. The HBV positive sera were excluded from this study. All abovementioned tests were achieved in virology department of AFLMR.

2.3 Detection of the parasitic infections

2.3.1 Preparation of Schistosoma mansoni and Fasciola hepatica antigens

Adult worms of *S. mansoni* were sourced from the Schistosome Biological Supply Program (SBSP) at Theodor Bilharz Research Institute, Giza, Egypt. The whole worms were homogenized in phosphate-buffered saline (PBS, 1:3 w/v). Centrifugation of the homogenate was carried out at 10000 rpm for 15 min. The supernatant containing the crude antigen was kept at -80 ºC until used.

Adult *Fasciola* sp. worms were collected from naturally infected sheep at El-Basaten slaughterhouse, Cairo, Egypt. The whole worms were collected from the common bile ducts, gall bladders, and main hepatic ducts of naturally infected sheep. All worms were washed three times with a normal saline solution and repeatedly washed with distilled water. They were incubated for 6 hours in 0.85% NaCl at room temperature to remove adherent host cells and empty intestinal caeca. Phosphate buffer (Ph 7.2) was added to the worms in the mortar in a ratio of 3:1 (v/w), then the worms were grinded manually for 5 min, and centrifuged for 10 min at 10000 rpm at 4ºC. The supernatant was taken, transferred to a clean tube, and kept at -80 ºC. The concentrations of protein

content of the homogenates of *S. mansoni* and *Fasciola* were determined by using Bio-Rad Kit for total protein measurement at a wavelength of 570–580 nm.

2.3.2 ELISA assay for detection antibodies of parasitic infections

The ELISA was performed to detect the parasitic infections with Schistosomiasis and/or Fascioliasis in HCV sera samples. Polystyrene 96-well plates were coated with a standardized quantity (1 µg/ml) of crude antigens (extracted from *Schistosoma mansoni* and *Fasciola* sp. adult worms), diluted in 0.05 M bicarbonate buffer (pH 9.6) and incubated overnight at 4 ◦C. The plates were washed three to five times with PBS/0.05% Tween-20 (PBST), followed by blocking with 1% (v/v) bovine serum albumin (BSA) (Win Lab., UK) in PBST (BSA-PBST) at room temperature for 2 h. After washing, 100 µl of each serum sample (diluted 1:100) in phosphate buffered saline (1×) was added to each well, and the plates were incubated at 37°C for 1 h. The plates were then washed and incubated 37 ◦C for 1 h with anti-human-IgG horseradish peroxidase-conjugated antibody (Sigma-Aldrich, St. USA) diluted in washing solution at 1:10000. This was followed by washing of the plates and addition of 100 µl of substrate solution, ortho-phenylenediamine (OPD) (Sigma) substrate, for 30 minutes into each well. The reaction was stopped by adding 50 µl per well of 4N sulfuric acid. The absorbance was estimated at 450 nm (ELx808, BioTek Instruments Inc, Vermont, USA).

All HCV serum samples were tested for the presence of anti-*T. gondii* antibodies, IgG using VITROS® 3600 immunological integrated System and Architect™ PLUS I1000SR immunoassay analyzer (ABBOTT company, Germany).

2.4.Clinical chemical determination

Vitros® 4600 chemistry system and VITROS 5600 integrated System (QuidelOrtho™, USA, https://www.quidelortho.com) were used to evaluate the liver function by estimating the serum levels of glutamate oxaloacetate aspartate aminotransferase (GOT), Glutamic-pyruvic transaminase (GPT), gamma-glutamyl transferase (GGT), total protein (TP), albumin (Alb), total bilirubin (T Bil), and alkaline phosphatase (ALK). Also, the kidney functions were evaluated by estimating the levels of creatinine, uric acid, urea, sodium (Na), and potassium (K) using the same system and its specific kits according to the instructions provided for each kit.

2.5. Statistical analysis

ANOVA and a non-parametric T-test (Mann-Whitney test) were used. The *P* value was considered significant when it was < 0.05 and highly significant when it was < 0.001, according to GraphPad Prism software program (version 8.0.2).

3. RESULTS

3.1. Detection of viral infection using PCR

From the 337 tested samples for HCV infections using the VITROS® 3600 Integrated System, it was found that 50 samples were free from both HCV and HBV (Hepatitis B virus) infections. The remaining sera samples (287) were individually tested for antibody titer reactivity against the antigens of *S. mansoni*, *F. hepatica*, and *T. gondii*.

3.2. Detection of parasitic infection using ELISA

Sera samples of HCV-infected patients were subjected to ELISA assay for detection of *S. mansoni*, *Fasciola*, and/or *Toxoplasma* antibodies. A total of 120 samples out of 287 samples of HCV-infected cases, with a percentage of 41.8%, were found to have developed *Schistosoma* antigen-specific antibodies. Among these cases, single schistosomiasis infections were recorded in 57 samples (47.5%), followed by samples from patients with co-infections: 31 (25.8%) with *Fasciola*, 24 (20%) with *Toxoplasma*, and 8 (6.6%) with both *Fasciola* and *Toxoplasma* (**Table 2)**.

Parallelly, a total of 99 sera samples (34.5%) from 287 HCV samples developed *F. hepatica* antibodies. Single infections (51 out of 99 51.5%), and mixed infections with *Schistosoma* and/or *Toxoplasma* were also recorded as, 31 (31.3%), 9 (9%), and 8 (8%) out of 99 samples, respectively (**Table 2)**.

Out of 287 samples from HCV-infected patients, 87 sera samples developed *T. gondii* antigen-specific humoral antibodies (IgG) with a percentage of 30.3 %. It was found that the largest percentage of serum samples (46 out of 87, 52.8%) from patients with toxoplasmosis were single infections. This was followed by samples from HCV-infected patients with companied infections, namely *Schistosoma* (24 out of 87, 27.5%), *Fasciola* (9 out of 87, 10.3%), and serum samples from patients with toxoplasmosis coinfected with both *Schistosoma* and *Toxoplasma* (8 out of 87, 9.1%) **(Table 2).**

Table 2: Parasitic infections frequencies among HCV patients

Parasite	No. of samples with single and mixed parasitic infections/ No. of inf. with specific parasite (%)				Total specific parasitic inf. (% from 287 HCV-infected cases)
	Fasciola **"F"**	***Toxoplasma*** **"T"**	***Schistosoma*** **"S"**	**S+T+F**	
Shcistosoma **(S)**	31/120 (25.8%)	24/120 (20%)	57/120 (47.5%)	8/120 (6.66%)	120 (41.80%)
Fasciola **(F)**	51/99 (51.51%)	9/99 (9%)	31/99 (31.3%)	8/99 (8.08%)	99 (34.49%)
Toxoplasma **(T)**	9/87 (10.34%)	46/87 (52.87%)	24/87 (27.58%)	8/87 (9.19%)	87 (30.31%)
Total mono-infected samples =57+46+51=154 Total double-infected samples=31+24+9=34 Total Trible-infected samples=8					

3.3. Group categories according to parasitic infection

Based on the collected data in **Tables (3 & 4),** the evaluated samples for parasitic infection were sorted into 9 groups according to the presence of HCV, mono-parasite, or co-infection. Each group was further categorized according to age and gender. The age categories were: <21 years old, 21-50 years old, and >50 years old. Group **(A)** was designated as free from HCV and parasitic infections, considered as healthy control (HCV-ve, P-ve). Group **(B) comprised** HCV positive samples but were free from any parasitic infection (HCV+ve, P-ve). Group **(C)** included HCV positive combined with *S. mansoni* infection (HCV+ve, S+ve). Group **(D)** HCV viral infection combined with *F. hepatica* infection (HCV+ve, F+ve). Group **(E)** consisted of individuals with HCV viral infection combined with the parasitic infection of *T. gondii* (HCV+ve, T+ve). Groups **(F), (G), (H),** and **(I)** were positively infected with HCV accompanied by mixed parasitic infections including *S. mansoni* and *F. hepatica* (HCV+ve, SF+ve)., *S. mansoni* and *T. gondii* (HCV+ve, ST+ve), *F. hepatica* and *T. gondii* (HCV+ve, FT+ve), *S. mansoni*, *F. hepatica* and *T. gondii* (HCV+ve, SFT+ve), respectively. Additionally, all the previous groups starting from B to I were further categorized referring to the viral load (qPCR results) into two subgroups: patients with **low viral** load (10^3 to 10^5 copies/ml) and patients with **high viral** load (> 10^5 copies/ml) group. (**Table 5**).

Table 3: Prevalence of single parasitic infections in HCV patients according to gender and ages

Group	A		B		C		D		E	
Age (year)	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)
< 21	-	1	6	2	6	-	4	-	1	2
	-	(2)	(9.83)	(3.2)	(10.52)	-	(7.84)	-	(2.17)	(4.34)
21 – 50	13	5	18	3	24	1	17	2	17	3
	(26)	(10)	(29.5)	(4.91)	(42.1)	(1.75)	(33.33)	(3.92)	(36.95)	(6.52)
> 50	28	3	27	5	18	8	22	6	18	5
	(56)	(6)	(44.26)	(8.19)	(31.57)	(14.03)	(43.13)	(11.76)	(39.13)	(10.86)
Total	41	9	51	10	48	9	43	8	36	10
(%)	82	18	83.6	16.3	84.2	15.7	84.3	15.6	78.2	21.7
A: healthy, B: HCV +ve, C: HCV & *Schistosoma* +ve, D: HCV & *Fasciola* +ve, E: HCV & Toxoplasma +ve. M: males, F: females										

Table 4: Prevalence of mixed parasitic infections in HCV patients according to gender and age.

Group	F		G		H		I	
Age (year)	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)	M (%)	F (%)
< 21	3	-	3	2	-	-	-	-
	(9.67)	-	(12.5)	(8.33)	-	-	-	-
21 - 50	9	6	3	5	1	2	2	1
	(29.03)	(19.35)	(12.5)	(20.83)	(11.11)	(22.22)	(25)	(12.5)
> 50	8	5	7	4	5	1	5	-
	(25.8)	(16.12)	(29.16)	(16.66)	(55.55)	(11.11)	(62.5)	-
Total	20	11	13	11	6	3	7	1
(%)	64.5	35.4	54.1	45.8	66.6	33.3	87.5	12.5
F: HCV, *Schistosoma* & *Fasciola* +ve, G: HCV, *Schistosoma* & *Toxoplasma* +ve, H: HCV, *Fasciola* & *Toxoplasma* +ve, I: (HCV, *Schistosoma*, *Fasciola* & *Toxoplasma* +ve) , M: males, F: females								

Table 5: Distribution of parasitic and HCV infections according to HCV viral loads

Group (No. of Patients)	Low viral load* (%)	High viral load** (%)

B (HCV+ve) (61)	**21 (35%)**	**40 (65%)**
C(*Schistosoma*) (57)	**24 (42.11%)**	**33 (57.89%)**
D (*Fasciola*) (51)	**15 (29.42%)**	**36 (70.58%)**
E (*Toxoplasma*) (46)	**19 (41.31%)**	**27 (58.69%)**
F (*S*+*F*) (31)	**9 (29.04%)**	**22 (70.96%)**
G (*S*+*T*) (24)	**6 (25%)**	**18 (75%)**
H(*F*+*T*) (9)	**1 (11.1%)**	**8 (88.8%)**
I(*F*+*S*+*T*) (8)	**4 (50%)**	**4 (50%)**
Chi square =5.608, *P*-value> 0.05, Non-significant		
* (10000-100000 copies/mL) ** (>100000 copies/mL) according to qPCR results		

We noted that the percentage of males in the groups with mono-parasitic infection was between 78.2% (*Toxoplasma*) and 84.3% (*S. mansoni or F. hepatica*). The percentages of these males (39-43.1%) were the highest over the age of 50 years in groups with mono-parasitic infection *Toxoplasma* and *F. hepatica*, respectively. However, in the group infected with schistosomiasis, the percentage of males in the age group 21-50 was the highest (42.1%). In general, the age group for patients under 21 was the lowest in the male percentage. Likewise, the group of patients infected with HCV was mostly from the age group over fifty (44.2% + 8.1% = 52.2%) (**Table 3**).

The same profile was identical in the patient groups with mixed parasitic infection in that the number of males was greater than the number of females. On the other hand, most of the patients infected with both *Schistosoma* and *Fasciola* (group F, 29% +19.3% = 48.2%) were from the age group 21-50, while the remaining patients with mixed parasitic infection belonged to the age group above 50 years (**Table 4**).

Most HCV patients in this study, whether infected with the virus alone or even co-infected with one or more parasites, had a high viral load (>10^5 copies/ml). However, the highest viral load was observed in patients with mixed parasitic infections involving both *Fasciola* and *Toxoplasma* (Group H, 88.8%). (**Table 5**)

3.4. Liver functions parameters

Tables (6 and 7) illustrate the tested parameters of the liver functions for all the examined groups including both low and high viral loaded sample categories, respectively. All low viral loaded samples had non-significant variation in GOT level in comparison with group A (-ve control) (*P*-value > 0.05) (**Table 6**). On the other hand, as illustrated in **Table (7),** high viral loaded samples of groups C (HCV +ve with *S. mansoni*), D (HCV +ve with Fascioliasis)

and I (HCV +ve with F, S, T) showed significant increases in the levels of GOT (*P*-value < 0.05). However, the other groups did not show significant difference from the negative control group (*P*-value > 0.05).

Table 6: Liver-specific serum parameters for the different groups with low viral loaded samples

Group / parameter	A (HCV-ve) M±SD	B (HCV +ve) M±SD	C (*Schistosoma*) M±SD	D (*Fasciola*) M±SD	E (*Toxoplasma*) M±SD	F (*S+F*) M±SD	G (*S+T*) M±SD	I (*F+S+T*) M±SD
GOT	43±20	34.95±20.7	53.36±35.6	50.86±27.9	52.47±33	51.13±29	50.8±28.8	34.5±3.69
GPT	31.24±15.3	45.3±20	47.75±30.37**	42.07±26.8	44.69±31.75	43.13±32.38	46±26.24	17.33±3.05*
TP	7.211±1.26	7.142±1.04	7.79±0.99	7.40±1.55	7.41±1.02	6.52±0.97	8±0.95	7.6±0.94
ALK	63.21±17.7	66.38±17.2	90.14±36.2**	81.58±38	91.61±35.17**	72.14±27.6	85±12.4**	97.75±41.1**
T Bil	0.615±0.2	0.862±0.4	1.468±1.6**	0.946±0.66*	0.883±0.72*	0.844±0.66*	1.217±1.12**	0.7±0.1
Alb	3.907±0.80	4.25±0.73	4.25±0.91	4.63±0.70	3.76±0.67	3.74±0.68	4.6±0.56	4.05±0.46
GGT	26.97±9.75	66.75±50.9***	93.13±53.5***	53.6±24.96***	62.08±35***	49.5±29.1**	49±34.48*	61.75±39.3***

GOT: glutamic oxaloacetic transaminase (normal level 8-45u/L)**, GPT:** Glutamic-pyruvic transaminase (normal range 7-56 u/L)**, TP:** Total proteins(normal range 6-8g/dL)**, ALK:** alkaline phosphatase (normal range 44-147IU/L)**, T Bil:** Total bilirubin (normal range 0.1-1.2 mg/dL)**, Alb:** Albumin(normal range 3.4-5.4g/dL)**, GGT:** gamma-glutamyl transferase (normal range 5-40U/L).

* *P*-value < 0.05, Significant ** *P*-value < 0.01, very significant *** *P*-value < 0.001 highly significant

Table 7: Liver-specific serum parameters for the different groups with high viral loaded samples

Group / Parameter	A (HCV-ve) M±SD	B (HCV +ve) M±SD	C (*Schistosoma*) M±SD	D (*Fasciola*) M±SD	E (*Toxoplasma*) M±SD	F (*S+F*) M±SD	G (*S+T*) M±SD	H (*F+T*) M±SD	I (*F+S+T*) M±SD
GOT	43±20	50.18±39.0	59.75±53.7*	55±32.6*	49.12±26.8	50.83±26	41.36±18.3	43.63±20.5	66.25±22.6*
GPT	31.24±15.3	62±61.2**	44.7±23.5*	55.2±27.1*	58.25±38**	47.57±26*	33.18±15.4	56.86±29**	57.5±20.36**
TP	7.21±1.26	7.39±0.91	7.7±1.27	7.3±1.27	7.6±0.91	6.98±1.3	7.67±1.07	7.58±1.55	8.24±0.47
ALK	63.2±17.8	84.38±53.7	98.82±66.8*	103.1±45.1**	109±78.2*	100.7±41.1**	81.3±28.4	91.63±41.1	90±36
T Bil	0.615±0.20	0.78±0.38	1.461±1.4**	1.06±0.86*	0.892±0.34*	0.958±0.46*	1.24±1.61**	1.07±0.57*	0.9±0.35*
Alb	3.90±0.8	4.13±0.66	4.40±0.69	4.1±0.64	4.25±0.7	4.07±1.15	4.28±0.7	4.36±0.9	3.95±0.7
GGT	26.97±9.75	69.26±54.2***	81.59±99.9***	81.11±66.1***	101.1±83.2***	56±30.1***	64.69±24.56***	59.67±23.8**	53.2±14.0***

GOT: glutamic oxaloacetic transaminase (normal level 8-45u/L)**, GPT:** Glutamic-pyruvic transaminase (normal range 7-56 u/L)**, TP:** Total proteins(normal range 6-8g/dL)**, ALK:** alkaline phosphatase (normal range 44-147IU/L)**, T Bil:** Total bilirubin (normal range 0.1-1.2 mg/dL)**, Alb:** Albumin (normal range 3.4-5.4g/dL)**, GGT:** gamma-glutamyl transferase (normal range 5-40U/L).

* *P*-value < 0.05, Significant	** *P*-value < 0.01, very significant	*** *P*-value < 0.001	highly significant

Regarding the liver enzyme GPT analysis, a non-parametric ANOVA test showed statistically high significant variations among all groups (*P*-value < 0.01). When using t-test (Mann-Whitney test) to compare each group with Group A, it was found that groups B (high viral loads), C (low viral load), E (high viral load), H (high viral load) and I (high viral load) showed highly significant increases in GPT levels (*P*-value < 0.01), while groups C (high viral load), D (high viral load) and F (high viral load) revealed slightly significant increases (*P*-value < 0.05). In contrast, all the remaining groups showed non-significant differences in GPT level in comparison with groups A and B (low and high viral loads) (*P*-value > 0.05**).**

All groups showed non-significant variations in both of Total protein and albumin serum levels.

In respect to the Alkaline phosphatase level, groups C, E, G, and I (with low viral loads) and groups D and F with high viral loads showed highly significant increases where the *P*-value < 0.01. However, groups C and E (high viral load) showed less significant increases in ALK level (*P*-value < 0.05). On the contrary, the serum level of GGT showed fluctuations in the degree of significant increase for all groups of low and high viral loaded samples in comparison with -ve control group **(Tables 6 & 7).**

In cases of serum level of total bilirubin, groups D, E, and F with low viral loads showed significant increases in comparison with group A (*P*-value < 0.05), while groups C and G showed a highly significant increase (*P*-value < 0.01). Likewise, the same groups with high viral loads in addition to groups G, H, and I showed significant increases with different degrees (*P*-values < 0.05 and 0.001), especially, group C which was co-infected with *Schistosoma* sp..

It was found that liver enzyme levels (GPT, GOT, Alk, and GGT) were more affected by the type (mono or mixed) or species of parasitic infections in HCV patients. Besides, most of the serological parameters of the liver in this study were significantly elevated with viral/parasitic infections, and these elevations increased in a stimulating manner in patients with high viral loads.

3.5. Kidney functions parameters

Tables (8 and 9) illustrate the tested parameters of the kidney functions for all the examined groups including low and high viral loaded samples categories, respectively. As shown in **Table (8)**, when comparing the serum creatine levels in patients with low viral loads

belonging to groups B, E, D, and F versus group A, there were less significant (Group B & E, *P*-value <0.05) and highly significant (Group D & F, *P*-value < 0.01) increases. Also, as described in **Table (9)**, samples of patients with high viral loads belonging to groups E and F showed highly significant increases in the creatine levels (*P*-values < 0.01% & < 0.001, respectively), whereas groups G and H showed less significant increases (*P*-value <0.05). However, no significant differences were observed in the remaining groups.

Uric acid analysis in all groups showed non-significant differences in comparison with group A, except for group F with low viral load **(Table 8), and** groups H and I with high viral loads (**Table 9**), where they showed the least significant increases in blood uric acid levels (*P*-value < 0.05).

In respect to blood level of urea, when compared with the corresponding data in the healthy group A, it was found that groups B, D, and F (with low viral load) showed very significant increases (*P*-value < 0.01), while the groups C and E showed the least significant increases (*P*-value < 0.05) (**Table 8**). On the other hand, in the high viral load groups, it was found that groups E and F exhibited highly significant increases (*P*-value < 0.001). Groups B, C, and D showed less significant increases (*P*-value < 0.05) (**Table 9**).

Concerning sodium (Na) levels, serum samples from patients with high viral loads (**Table 9**), except for groups G and I, showed significant decreases when compared with group A (*P*-value < 0.05). However, no significant differences were observed in the remaining groups with low viral load (**Table 8**). Besides, potassium (K) levels in patients' blood samples in groups B and I (with low viral load, **Table 8**) showed significant increases (*P*-value < 0.01), likewise, groups C, D, F, G and H (with high viral loads), showed relatively high significant elevations in K levels in comparison with the healthy group A (*P*-value < 0.01). However, the remaining groups did not show significant differences from the healthy group A (**Tables 8 and 9**).

It was observed that most kidney parameters (Creatinine, Urea, Na, K) in patients with HCV with a high viral load and suffering from parasitic infections (*Schistosoma*, *Fasciola* and *Toxoplasma*) were more affected than the parameters (Creatinine and Urea) in patients with a low viral load.

Table 8: Kidney-specific serum parameters for the different groups with low viral loaded samples.

Group / Parameter	A (HCV-ve) M±SD	B (HCV +ve) M±SD	C (*Schistosoma*) M±SD	D (*Fasciola*) M±SD	E (*Toxoplasma*) M±SD	F (*S*+*F*) M±SD	G (*S*+*T*) M±SD	I (*F*+*S*+*T*) M±SD
Creatinine	0.75±0.158	2.75±2.9*	1.81±2.6	2.25±2.7**	2.36±2.9*	2.73±2.6**	0.86±0.16	1.97±2.0
Uric acid	5.01±1.2	5.98±1.53	5.68±1.22	4.98±1.21	5.22±1.53	6.92±2.55*	5.15±1.73	5.4±0.77
Urea	32.77±15.2	72.65±60.0**	48.19±30.2*	60.15±37.7**	49.75±25.6*	60.29±32.4**	32±3.87	50.5±26.8
Na	134±6.17	127.2±11.5	135±3.432	125.9±11.2	126±14.33	126.1±12	128.2±18.3	128±12.4
K	4.41±0.59	5.31±1.68**	4.98±1.08	4.86±0.53	4.8±0.85	4.77±0.53	4.94±1.03	5.45±2.4**

Creatinine (normal range 0.6-1.3mg/dL), **Uric acid** (normal range 3.5-7.2mg/dL), **Urea** (normal range 5-20mg/dL), **Na** (normal range 136-145mmol/L), **K** (normal range 3.5-5.2mmol/L).
* *P*-value < 0.05, Significant ** *P*-value < 0.01, very significant *** *P*-value < 0.001 highly significant

Table 9: Kidney-specific serum parameters for the different groups with high viral loaded samples.

Group / Parameter	A (HCV-ve) M±SD	B (HCV +ve) M±SD	C (*Schistosoma*) M±SD	D (*Fasciola*) M±SD	E (*Toxoplasma*) M±SD	F (*S*+*F*) M±SD	G (*S*+*T*) M±SD	H (*F*+*T*) M±SD	I (*F*+*S*+*T*) M±SD
Creatinine	0.75±0.15	1.41±1.9	1.62±2.6	1.841±2.33	2.50±2.9***	3.12±2.9***	2.44±2.8*	2.17±2.6*	0.95±0.31
Uric acid	5.01±1.23	5.61±1.26	5.59±1.24	5.52±1.27	5.28±1.25	5.23±1.78	4.98±1.2	6.2±0.89*	6.52±1.2*
Urea	32.7±15.2	50.23±43.8*	49.59±34.6*	50.29±39.0*	60.24±38.0***	63±28.5***	49±23.1	43.43±30.9	33.5±17.3
Na	134±6.17	129.6±9.2*	129.8±8.4*	129.2±8.83*	129±7.9*	130.1±7.47*	131.2±9.08	127.3±7.9*	134±4.35
K	4.41±0.59	4.68±0.83	5.12±1.05**	5.49±1.75**	4.67±0.67	5.17±0.99**	5.0±0.728**	6.16±2.01**	4±0.65

Creatinine (normal range 0.6-1.3mg/dL), **Uric acid** (normal range 3.5-7.2mg/dL), **Urea** (normal range 5-20mg/dL), **Na** (normal range 136-145mmol/L), **K** (normal range 3.5-5.2mmol/L).
* *P*-value < 0.05, Significant ** *P*-value < 0.01, very significant *** *P*-value < 0.001 highly significant

4. DISCUSSION

An ELISA assay was carried out to detect three parasitic infections (schistosomiasis, fascioliasis, and toxoplasmosis) in all tested samples of HCV patients. ELISA is a powerful immunological tool in estimating parasitic infection in HCV patients' samples **(9)**. According to gender, the present study showed that the prevalence of all parasitic infections in male HCV patients was higher than in females with non-significant variations. This agreed with schistosomiasis study achieved by **Chisango (10)** who suggested that males are more likely to be infected with schistosomiasis than females. One plausible explanation is that men spent more time fishing and practicing irrigation farming, so they are at an increased risk of exposure to contaminated water bodies (**11,12)**. However, the distribution of fascioliasis by sex shows variable results. A large study by Parkinson **(13)** in the Bolivian Altiplano involving almost 8000 subjects of all ages failed to find a significant association with sex. In a study including over 21,000 children in Egypt **(14)** reported that females had a significantly higher prevalence of fascioliasis and passed more eggs in the stool than males. Nonetheless, it should be emphasized from the findings of the present study that men represented the majority of the clinic's patients. Concerning *Toxoplasma* infection, **(15)** reported that the rural residence and increased age were found as risk factors for toxoplasmosis, whereas gender was not found to be a significant factor.

In the current study, the age groups of the screened individuals were represented in three age categories. The distribution of parasitic infections among HCV patients according to age showed that the most infected samples were among patients aged more than 50 years old, where the percentages of monoparasitic infections were more prominent than the mixed infections (26/175, 28/175, and 23/175 infected with *S. mansoni, Fasciola, Toxoplasma,* respectively). This is compliant with the earlier study carried out by **Raso (16).**

This observation can be explained, firstly, as some immunological factors might change with advanced age; hence, older individuals are less protected against parasite challenges **(17).** Secondly, a shift in occupational activities at a later age could lead to an increase in water contact, potentially causing the second peak (especially in *S. mansoni* and *Fasciola* infections). Additionally, it is possible for the patient's reluctance to undergo medical examinations at the first symptoms of the parasitic symptoms until complications appear at an advanced age.

GPT, TBIL, and GGT were significantly elevated in most experimental groups, especially in highly viral loaded patients, in contrast, no significant changes were recorded in serum levels of albumin or total protein along the study. Some studies agreed with our results **(18)**, Possible causes might be damage to the membrane of the liver, hepatic manifestations originating from the deposition of viral infection or parasite inside the small vessels of the liver. This can lead to an intense inflammatory response and subsequent functional changes, a situation which presumably may be responsible for the significant elevation of these circulating liver enzymes. Many studies reported elevated levels of GOT and GPT as well as increased enzyme activities in the sera of samples of HCV patients **(19).**

Regarding bilirubin, a result of the body's regular process of breaking down red blood cells, bilirubin is the main bile pigment that, when high, causes the yellow discoloration of the skin, sometimes referred to as jaundice. Numerous types of liver or biliary illnesses can result in high bilirubin levels. When liver cells are damaged by hepatitis, the liver may release both indirect and direct bilirubin into the bloodstream, resulting in higher levels. Similarly, the host liver is harmed during the *F. hepatica* invasion. Hepatic tissue is broken down by parasite, leading to significant parenchymal loss, severe hemorrhagic lesions, and immune responses. Besides, juvenile flukes that migrate are the cause of mechanical liver injury **(20)**. In HCV patients with schistosomiasis, liver function gets worse more quickly, often resulting in severe, irreversible periportal fibrosis and a faster progression to end-stage liver disease **(21).** In this study, we found that infection with schistosomiasis caused the highest increase in bilirubin than other infections, due to the possible role of surface egg antigens (SEA) in inhibiting some important genes in bilirubin metabolism, such as UGT1A1**(22).**

Gamma-glutamyl transferase, or GGT, is an enzyme that mainly exists in the liver. It has long been thought that the process of hepatic destruction in chronic viral hepatitis determines variations in GGT activity **(23)**. Some authors have proposed that these alterations could be caused by damage to the bile ducts, the advancement of liver disease, or an inadequate reaction to interferon (IFN) treatment **(24).** The exact significance of the GGT change often seen in patients with chronic HCV infection is still unknown, despite a few reasons being proposed for it **(19).**

In line with Giannini **(25),** it was observed that the elevated GGT levels were linked to the presence of bile duct lesions in individuals with chronic HCV. However, the levels of HCV viral

load and GGT in the serum did not significantly correlate. These findings were consistent with those of **(26)**, who reported that there was no correlation between serum GGT elevation and either the HCV genotype or serum HCV RNA titer.

The relationship between *T. gondii* infection and liver disease was assessed by Babekir **(27)** using the Mantel-Haenszel risk ratio (RRMH), Rho-Scott chi-square bivariate analyses, design-based t-tests, and linear and logistic regression models. It was observed in the present data that the patients co-infected with the parasite *Toxoplasma* and the C virus have greater values of GGT, possibly due to the fact that parasite can cause DNA damage, shape distortion, and disruptions in the hepatocyte's metabolic activities when it invades **(28).** The quantity of hepatic stellate cells (HSCs) and *T. gondii* antigens also significantly correlate, suggesting an active involvement of HSCs in liver pathology and the pathobiology of *T. gondii*-related hepatitis.

On the other hand, the parasites (*Schistosoma* and *Fasciola*) are known to be in the liver of the host and to induce pathological alterations that lead to necrosis, granuloma, and hepatomegaly **(29)**. Infestation of *S. mansoni* can cause portal hypertension, liver fibrosis, and possibly even an increase in liver enzymes such as GGT **(30).** After *Fasciola* enters the bile duct, damage to the bile duct epithelium results in the release of GGT into the bloodstream, which raises the GGT level in serum **(31).** This significant rise in GGT level is linked to bile duct injury and cholestasis **(18).** The possible causes might be due to hepatic manifestations originating from the deposition of parasite/viral infection inside the small vessels of the liver, which can lead to an intense inflammatory response and subsequent functional changes, a situation that presumably may be responsible for the significant elevation of these circulating liver enzymes.

In the present study, we found that there is a varying signification between parameters of kidney function (creatinine, uric acid, urea, Na and K) among the viral samples when compared with group A (HCV-ve). A significant medical burden for patients with chronic renal disease is HCV infection. Patients with chronic kidney illness are more likely to contract HCV infection even though HCV infection itself can induce chronic kidney disease (CKD), primarily mixed cryoglobulinemia, glomerulonephritis, and membranoproliferative glomerulonephritis (MPGN) **(32).** A larger HCV viral load has the potential to induce more serious glomerulopathy. Patients with CKD have weakened immune systems, which increases their risk of infection **(33).** It is unclear what exactly causes the reduction in renal function associated with *Schistosoma* infection. Several theories have

been put out, including immune-mediated glomerular and tubular disease, changes in the renal microcirculation, fluid loss through a variety of pathways, and mechanical obstruction by infected erythrocytes **(34)**. It is possible that immune complex deposition has a role in the pathophysiology of renal involvement **(35).**

There are similarities in the pathophysiology of the glomerular lesion in schistosomiasis and other parasitic illnesses, like malaria. In schistosomiasis, the glomerular lesion is of an immunological character. Human and animal sera infected with *S. mansoni* include antigens from the parasite that appear to be linked to glomerulopathy **(36)**. Besides, the infected humans and animals have also been shown to have antibodies against the parasite, which appear to be connected to the onset of glomerular damage **(37)**. The majority of the isolated circulating antigens involved in the pathophysiology of glomerulopathy originate from the adult parasite's digestive tract **(38)**.

The presence of *T. gondii* IgG antibodies was used to measure *T. gondii* exposure, while CKD biomarkers were used to determine the state of the disease. Multivariable regression models were employed **(27)** to examine association between CKD biomarkers and *Toxoplasma* infection while controlling clinical, anthropometric, behavioral, and sociodemographic variables frequently linked to renal failure. The findings revealed that participants with positive *T. gondii* IgG antibodies had noticeably higher levels of CKD biomarkers.

It is unknown how exposure to *T. gondii* harms the renal system. Prior research has indicated that *Toxoplasma* infection causes cells to produce more reactive oxygen species (ROS) and nitric oxide, which subsequently results in oxidative stress **(39)**. This oxidative stress, which is associated with renal failure, sets off an initial inflammatory response that is mediated by the transcription factor nuclear factor-κB (NF-κB), proinflammatory mediators, tumor necrosis factor (TNF-alpha), interleukin (IL-1b), and proinflammatory mediators. Extracellular matrix is synthesized as a result of increased transforming growth factor beta (TGF-beta) production during the later stages of inflammation **(40)**. Therefore, inflammation and consequent tissue damage are the mechanisms via which the long-term effects of oxidative stress on kidney tissues are conveyed, ultimately resulting in organ dysfunction. We also note in our results that the group that is the co-infection of samples from patients with C virus and the *Toxoplasma* parasite has a higher value than the rest of the groups.

Generally, Na levels decreased in most HCV-patient groups with high viral loads. A common side effect of advanced cirrhosis is hyponatraemia, which is caused by a reduction in the renal ability to eliminate solute-free water. This leads to an excessive retention of water compared to sodium, resulting in lowered serum sodium concentration and hypo-osmolality. The primary pathogenic mechanism linked to circulatory dysfunction that causes hyponatraemia is a non-osmotic hypersecretion of arginine vasopressin (AVP), also known as antidiuretic hormone, from the neurohypophysis. In cirrhosis, hyponatraemia is linked to higher morbidity and death rates **(41).** The generation of free radicals and modifications to liver antioxidant levels during host-parasite contact lead to fibrosis and other metabolic disorders **(42).** Dendritic cells have also been demonstrated to upregulate and utilize voltage-gated potassium (KV) channel activity for cytokine production, major histocompatibility complex (MHC) class II expression, chemotaxis, and phagocytosis **(43).** Additionally, nitric oxide (NO) generation in macrophages in response to antimicrobial agents requires potassium channel activation **(44).**

Urea levels were significantly elevated in groups with low viral loads (B (HCV+), D (HCV+F), and F (HCV+S+F)). In patients with high viral loads, urea levels were highly significantly elevated in groups E (HCV+T) and F (HCV+S+F). The most common cause of elevated urea levels is abnormal urea production or excretion. One of the liver's primary roles in maintaining the body's overall nitrogen balance is ureagenesis, which deals with the ultimate, irreversible conversion of amino nitrogen to urea nitrogen **(45).**

Purine metabolism, which derives from both endogenous and external sources, culminates in uric acid (Hyperuricemia) **(46).** It is catalyzed by xanthine oxidase (XO) and processed by the muscles, intestines, and liver. Roughly two thirds of uric acid are eliminated by urine, with the other third being expelled through feces **(47).** Because female individuals have greater plasma estrogen levels than male patients, there is a possibility that this could lead to a better urate clearance in urine, resulting in lower serum uric acid levels. Numerous additional risk factors have also been reported to be connected to hyperuricemia **(48)**. Patients with chronic HCV are thought to be a unique group with metabolic disorders. Similar risk factors for hyperuricemia were found in both the general population and HCV patients in the current study.

The harmful effects of *Toxoplasma* on the kidney may be the cause of the rise in urea concentration. These effects include decreased urea excretion from the body and an increase in its

blood level. The afflicted mice's kidneys contained *Toxoplasma* cysts, which caused several pathological alterations in their organs. Kidney damage from *Toxoplasma* infection can result in increased protein excretion in the urine and hypoalbuminemia **(49)**.

Generally, direct parasite damage, immunological phenomena such as immune complex deposition and inflammation, and systemic symptoms such as hemolysis, hemorrhage, and rhabdomyolysis are the processes behind kidney injury associated with parasitic diseases **(50).**

In conclusion to the above, it was found that the sera levels of liver markers (GPT, total bilirubin, and GGT) and kidney parameters (creatinine, urea, Na, and K) were more affected by the type (mono or mixed) or species of parasitic infections, and that most of these biochemical parameters in this study were significantly elevated with viral/parasitic infections. Regarding the effect of parasitism on HCV patients, it was found that GGT has remarkably increased in HCV patients with *Schistosoma* and *Toxoplasma* infections in the low and high viral load groups, respectively. While GPT clearly has decreased in HCV patients with triple and double (*Schistosoma*/ *Toxoplasma*) parasitic infections in the low and high viral load groups, respectively. Alkaline phosphatase has significantly increased in HCV patients with triple parasitic and *Toxoplasma* infections in the low and high viral loaded groups, respectively. In a striking way, total bilirubin has increased in HCV patients (low and high viral load groups) with single infection (*Schistosoma*). Creatinine has decreased in HCV patients (low viral load) with double parasitic infections (*Schistosoma*/ *Toxoplasma*), while it has remarkably increased in HCV patients (high viral load) with double parasitic infections (*Schistosoma*/ *Fasciola*). Moreover, urea has remarkably decreased in HCV patients (low viral load) with double parasitic infections (*Schistosoma*/ *Toxoplasma*), and as well as in HCV patients (high viral loaded) with tribble parasitic infections. Besides, Also, the highest viral load in experimental groups was in patients with mixed parasitic infections with both *Fasciola* and *Toxoplasma* (88.8%). Exceptionally, the total protein and albumin show non-significant changes in their serum levels either in patients with low or high viral load. Moreover, the urea, and potassium, showed decreasing changes in their levels in patients with the high viral loads. It is an urgent need to conduct studies for a deeper understanding of the metabolic interactions in the human body in the case of parasitic infections with the presence of any viral infection (as HCV), especially when the organ is a common target for both pathogens.

Declarations

Authors' contributions

Study concept and design: Hoda A. Taha, Marwa M. Aboueldahab, and Ahmed H. Nigm developed the original idea and the protocol. Administrative, technical, and material support: Maryam M. S. Garas, Hoda A. Taha, and Marwa M. Aboueldahab. Analysis and interpretation of data: Hoda A. Taha, and Marwa M. Aboueldahab and Ahmed H. Nigm. Drafting of the manuscript: Hoda A. Taha, Ahmed Nigm and Maryam M. S. Garas. Critical revision of the manuscript for important intellectual content: Hoda A. Taha, Marwa M. Aboueldahab and Ahmed H. Nigm. Statistical analysis: Hoda A. Taha and Maryam M. S. Garas.

Conflict of interest

The authors declare that they have no competing financial interests or personal relationships that could potentially influence the outcome of this research study. The study was not funded by any company or for-profit organization.

Ethics

All human procedures and experimental protocols concerning this work were reviewed and approved by the Scientific Research Committee of Egypt Center for Research and Regenerative Medicine (ECRRM), Egypt. (OHRP Reg. IORG0010559 – IRB00012517 – MOHP: RHDIRB2021020101-220124-01UC-MD-No.0124).

This cross-sectional study was performed at Armed Forces Medical Research Laboratories and Blood Bank (AFLMR) during the period between December 2020 and March 2021. Patients who were confirmed to be infected with HCV were included in this study. They were randomly sampled, including males and females with an age range between 20 to more than 50 years old. 50 normal subjects free from HCV and any parasitic infections were used as controls. Patients with hepatitis B were excluded from this study.

Data Availability

All data generated or analyzed during this study are included in this published article.

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