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Prevalence of Human Herpes Virus-6 (HHV-6) among Chronic HCV Patients

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Original Research Article

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Abstract: Herpes viruses as CMV, EBV and HHV-6 are the most common cause severe morbidity and mortality in immunocompromised individuals. Reactivation of the virus is seen during periods of down-regulation of the immune system, such as co-infection with other pathogens. So, this study was conducted to detect the presence of Human herpes virus-6 infection in patients with chronic hepatitis C virus (HCV) infection and to point out the effect of HHV 6 -HCV co-infection on Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) liver enzymes. Nested polymerase chain reaction was carried out on extracted DNA from sera of both groups with hepatitis C and control group. Serological investigations were carried out as well. Fifty-three serum samples with HCV-RNA were examined, (25%) were positive HHV-6 DNA, (82%) were positive for HHV-6 IgG antibodies and (22%) were positive for HHV-6 IgM antibodies; among control specimens (18.5%) were positive for HHV-6 DNA infection, (61%) were positive for HHV-6 IgG antibodies and (14.5%) were positive for HHV-6 IgM antibodies. Moreover, the activity levels of ALT and AST liver enzymes were significantly higher in HHV-6 positive patients than that of HHV-6 negative patients. This indicates to the prevalence of HHV-6 infection is common in chronic HCV patients, that increase the severity of liver inflammation.

Keywords: Herpes viruses, HHV-6, Human herpes virus-6, HCV.

INTRODUCTION

Human herpes virus 6 (HHV-6) is a human B-lymphtropic virus that was identified as a member of the β-herpesvirus group [1]. The importance and the interest of HHV-6 as a pathogen have increased over the past two decades. (HHV-6) is the most common cause severe morbidity and mortality in immunocompromised individuals [2].

A primary HHV-6 infection is followed by a life-long persistence of the virus in a latent state, and reactivation may occur later in life [3]. Therefore, reactivation of the virus is seen during periods of down-regulation of the immune system, such as drug treatment and illness-related stress, or during on-going activation of the immune system such as inflammatory diseases, or co-infection with other pathogens [4].

HHV-6 can infect virtually all organ tissues, but manifestations of organ involvement generally include symptoms from the liver, salivary glands and the CNS [5].

The cytopathic potential of HHV-6 in human liver cells was analyzed in cell culture and in tissue sections from patients with HHV-6 hepatitis, and it was concluded that HHV-6 can cause direct liver paranchymal damage by efficient cytolytic infection of hepatocytes [6, 7]**.** HHV-6 hepatitis occurs as part of disseminated HHV-6 infection. It occurs mainly among liver or kidney transplant recipients or immunosuppressed persons [8, 9]. Mild-moderately elevated levels of transaminases and various histopathological changes of the liver were encountered in these patients [10].

This study aimed to investigate the co-infection of human herpes virus 6 (HHV-6) (antibodies and DNA) in sera samples from patients positive and negative for HCV infection to study the effect of HHV-6-HCV co-infection on Liver function.

MATERIALS AND METHODS

Study population

The present study included of eighty four cases (48 female and 36 male, age range 18:64; mean age 37.62±10.03 years). Fifty three patients with positive HCV RNA (mean age: 39.92± 9.34; range: 25- 64; 31 females, 22 males) and Thirty one cases without viral hepatitis (control group) (mean age: 35.32± 10.72; range: 18 - 57; 17 females, 14 males).

Detection of HCV RNA

RNA was extracted from 200µl of serum specimen using the acid guanidium thiocyanate-phenol-chloroform method [11].

Primer sets used in the detection of HCV RNA were selected from the highly conserved 5'- untranslated region (UTR) of the HCV genome. p: 5' GGTGCACGGTCT ACGAGACCTC 3' - P2 forward primer: 5' AACTACTGTCTTCACGCAGAA 3' - P3 reverse primer: 5' TGCTCATG GTGCACGGTCTA 3'- nested reverse primer P4: 5'ACTCGGCTAGCAGTCTCGCG 3' and forward primer P5: 5' GTGCAGCCTCCAGGACCC 3' (Promega, USA).

The nested PCR amplification was done in a volume of 50 µl; and the PCR protocol consisted of a reverse transcription step at 59 ◦C for 60 min by using 20 U of cloned Avian Myloblastosis Virus (AMV) reverse transcriptase and 1µl primer (p1). First round amplification was done by using (P2) forward primer and (P3) reverse primer. The second round amplification was done similar to the first round, except for use of the nested reverse primer (P4) and forward primer (P5), the products of nested PCR were analyzed on 2% agarose gel electrophoresis.

Serological analysis of HHV-6 infection

HHV-6 IgM and HHV-6 IgG antibodies were estimated by the enzyme-linked immunosorbent assay (ELISA) technique using commercially available HHV6 IgM and IgG Kits, (MyBiosensor Diagnostic HHV6 IgM ELISA Kit, USA, and MyBiosensor Diagnostic HHV6 IgG ELISA Kit, USA). Tests were done according to the manufacturer instructions.

Detection of HHV-6 DNA

DNA was extracted from 300 µl serum sample using Wizard® DNA purification mini kit, Promega (Madison, USA), following the instructions of the manufacturer. HHV-6 DNA was detected using the primers of large-tegument protein gene of HHV-6. 3 µl of the DNA extract from the sample was added to 20 µl of PCR mixture 1 µl of each primers F1: 5` CGCAGAGACATATCGTTCCGATGG 3` and R1: 5`AGAACCGTCGCATCAATTACTCGC 3` (Bioneer, USA). 2µl of the 1st PCR product were used in a nested-PCR containing the same conditions as mentioned above, using internal primers F2:5`AATAGGAGCCTTGCTGGTCAGAAC3` and R2:5`CCTGGAACCCCACAAAACCTAACG 3` (Bioneer, USA). Amplification products (nested-PCR products) were visualized after electrophoresis on 2% agarose gel stained with ethidium bromide.

Biochemical Analysis

Biochemical tests, including Alanine amino transferase (normal range, 40 U/L) and Aspartate amino transferase (normal range, 38 U/L) levels were done on all collected samples with commercially available Flex ALAT (GPT) and ASAT (GOT) Kits (Siemens Healthcare Diagnostic Inc., USA). Levels of liver enzymes were measured as described by the manufacturer.

RESULTS

Using nested PCR techniques (Fig-1) we can classify the all study cases into two groups: The first group (Patient group) included 53 cases of HCV positive RNA, whose ages ranged from 25 to 64 years and the mean was 40±9.34 years. They were 22 male representing 41.5% and 31 female representing 58.5% and the second group (Control group) included 31 cases of HCV negative RNA, whose ages ranged from 18 to 57 years and the mean was 35.32±10.72 years. They were 14 male representing 45.2% and 17 female representing 54.9%.

Results of ages represented as mean ± SD and sexes expressed as N= numbers, % = percentage in patient and control groups. HCV RNA was measured by RT-PCR

Table-1: Age and Sex in patients and controls

Variables \ Groups		Patient group (Positive HCV RNA) N= 53	Control group (Negative HCV RNA) N= 31
Age	mean	40±9.34	35.32±10.72
Male	N	22	14
	%	41.5%	45.2%
Female	N	31	17
	%	58.5%	54.9%

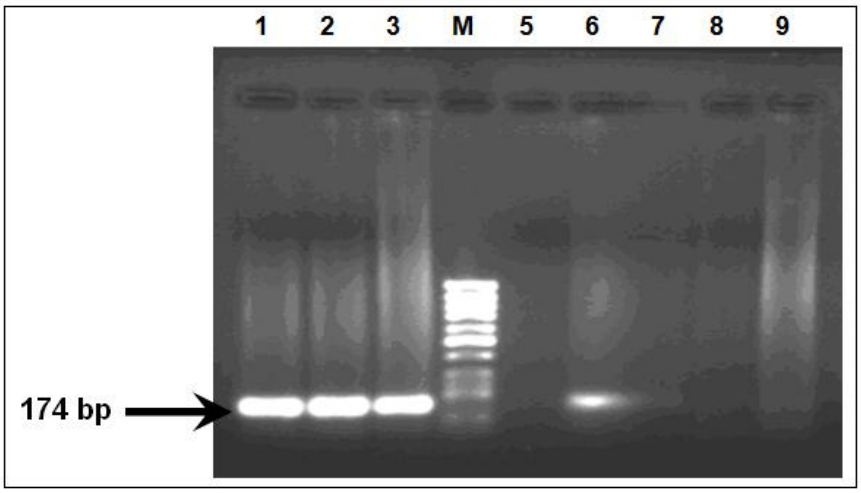

Fig-1: RT nested PCR results of serum samples lane 1, 2, 3 and 6 were positive for HCV RNA while lanes 5, 7, 8 and 9 were negative for HCV

Detection of HHV-6 IgG and HHV-6 IgM antibodies in patient group

In patient group: 13 out of 53 (24.5%) were positive for HHV-6 IgM antibodies, whose ages ranged from 38 to 61 years and the mean was 46.31±6.61 years. They were 6 male (46.2%) and 7 female (53.8%), 46 cases out of 53 (86.8%) were positive for HHV-6 IgG antibodies, whose ages ranged from 25 to 64 years and the mean was 39.72± 9.33 years. They were 21 male representing (45.7%) and 25 female representing (54.3%) (Table-1**).**

Table-1: The percentage of HHV-6 IgM & IgG among different cases of patient group

Variables \ Groups		HHV-6 IgM		HHV-6 IgG	
		positive	Negative	Positive	Negative
Age	Mean	46.31±6.61	37.85±9.22	39.72± 9.33	41.29±10.06
Male	N	6	16	21	1
	%	46.2%	40%	45.7%	14.3%
Female	N	7	24	25	6
	%	53.8%	60%	54.3%	85.7%
Total = 53	N	13	40	46	7
	%	24.5%	75.5%	86.8%	13.2%

Detection of HHV-6 DNA in patient group

According to nested PCR results in patient group (Fig-2), 20 cases out of 53 (37.7%) were positive for HHV-6 DNA, whose ages ranged from 25 to 61 years and the mean was 44±8.4 years. They were 8 male (40%) and 12 female (60%) (Table-2). Of these twenty cases, 17 cases representing (85%) were positive for HHV-6 IgG antibodies, (mean age: 44.08±8.85 years; range: 25:61 years, 7 male and 10 female) and 13 cases representing (65%) were positive for HHV-6 IgM antibodies, (mean age: 46.31±6.61 years; range: 38: 61 years, 6 male and 7 female).

Table-2: The percentage of positive or negative HHV-6- DNA among different cases of patient group

Variables \ Groups		HHV-6 DNA(+)	HHV-6 DNA(-)
Age	Mean	44±8.4	37.49±9.13
Male	N	8	14
	%	40%	42.4%
Female	N	12	19
	%	60%	47.6%
Total = 53	N	20	33
	%	37.7%	62.3%

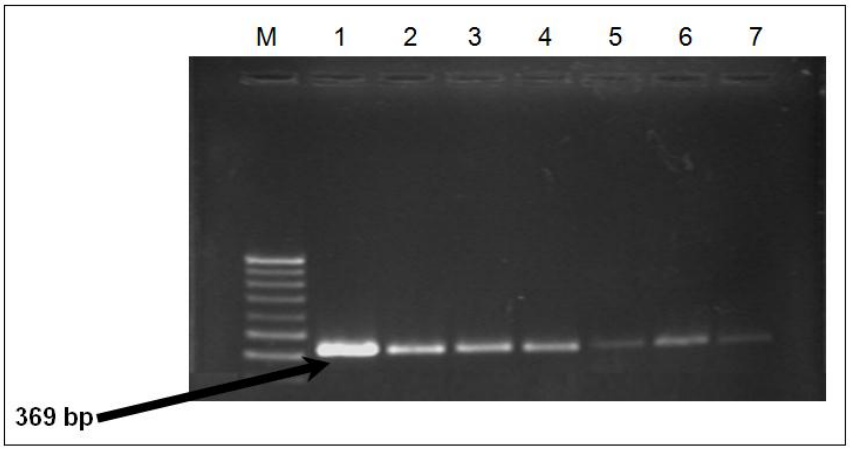

Fig-2: Nested PCR results of HHV-6 DNA in serum samples

Detection of HHV-6 IgG and IgM antibodies in Control group

In Control group, 5 out of 31 cases (16.13%) were positive for HHV-6 IgM antibodies, whose ages ranged from 18 to 30 years and the mean was 26.6±4.93 years. They were 3 male (60%) and 2 female (40%). although, 20 cases out of 31 (64.5%) were positive for HHV-6 IgG antibodies, whose ages ranged from 18 to 57 years and the mean was 36.8 ± 11.99 years. They were 9 male representing (45%) and 11 female representing (55%) (Table-3).

Table-3: The percentage of HHV-6 IgM & IgG among different cases of control group

Variables \ Groups		HHV-6 IgM		HHV-6 IgG	
		positive	negative	Positive	Negative
Age	Mean	26.6±4.93	37±10.76	36.8±11.99	32.64±7.67
Male	N	3	11	9	5
	%	60%	42.3%	45%	45.45%
Female	N	2	15	11	6
	%	40%	57.7%	55%	54.54%
Total = 31	N	5	26	20	11
	%	16.13%	83.87%	64.5%	35.5%

Detection of HHV-6 DNA in Control group

In control group, 7 cases out of 31 (22.58%) were positive for HHV-6 DNA, whose ages ranged from 18 to 41 years and the mean was 29.43 ±6.8 years. They were 5 male representing (71.4%) and 2 female representing (28.6%). Of these seven cases, 6 cases representing (85.7%) were positive for HHV-6 IgG antibodies, (mean age: 29.5±7.44 years; range: 18:41 years, 4 male and 2 female) and 5 cases representing (71.42%) were positive for HHV-6 IgM antibodies, (mean age: 26.6± 4.93 years; range: 18: 30 years, 3 male and 2 female) (Table-4).

Table-4: The percentage of positive or negative HHV-6- DNA among different cases of Control group

Variables \ Groups		HHV-6 DNA(+)	HHV-6 DNA(-)
Age	Mean	29.43 ± 6.8	37.04± 11.14
Male	N	5	9
	%	71.4%	37.5%
Female	N	2	15
	%	28.6%	62.5%
Total = 31	N	7	24
	%	22.58%	77.42%

Estimation of ALT & AST activity levels among Study groups

In patient group serum ALT levels (mean: 82.64±20.6 IU/L) were higher than that of control group (mean: 38.29± 11.38 IU/L), also serum AST levels of patient group (mean: 79.18± 21.69 IU/L) were higher than that of control group (mean: 35.65± 11.46 IU/L) (Table-5).

Table-5: Serum ALT& AST levels of Study population

Groups / Variables		Patient group (positive HCV patients)	Control group (negative HCV patients)
ALT (IU/L)	Mean	82.64±20.6	38.29± 11.38
AST (IU/L)	Mean	79.18± 21.69	35.65± 11.46

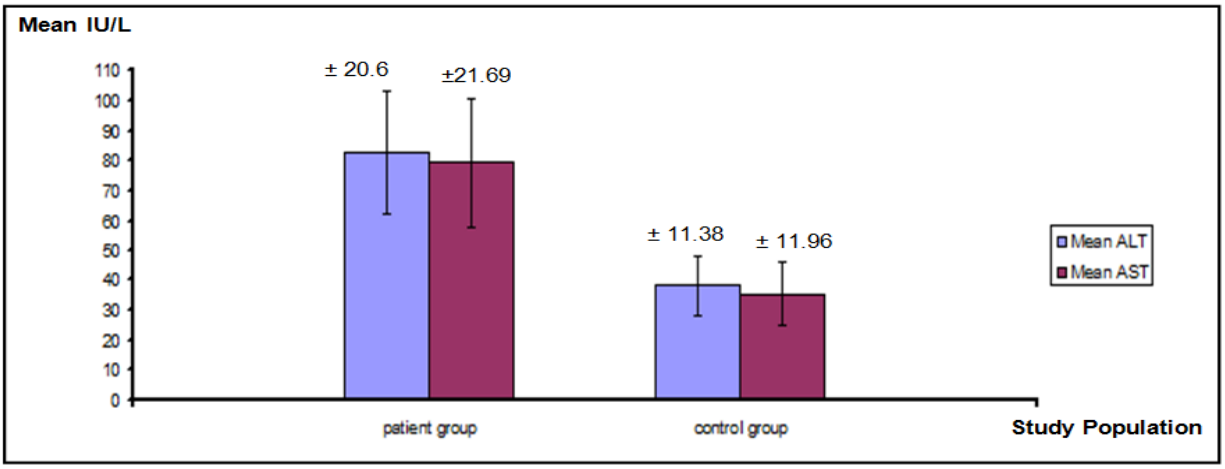

Fig-3: The mean ALT and AST activity levels of patient and control groups

ALT and AST activity levels in patient group (+ve HCV RNA)

In HHV-6 IgM positive patients, serum ALT level (mean: 95.69±29.50 IU/L) was higher than that of HHV-6 IgM negative patients (mean: 78.4±19.92 IU/L) and also, serum AST level of HHV-6 IgM positive patients (mean: 92.31±29.54 IU/L) was higher than that of HHV-6 IgM negative patients (mean: 74.93±16.82 IU/L) (Table-6).

Table-6: Detection of ALT& AST activity Levels in HHV-6 IgM patient group

	Positive HHV-6 IgM patients	Negative HHV-6 IgM patients	Statistics	
			T-test	P-value
Mean ALT (IU/L)	95.69±29.50	78.4±19.92	-99.82	0.0001
Mean AST (IU/L)	92.31±29.54	74.93±16.82	-10.06	0.01

In HHV-6 DNA positive patients, serum ALT level (mean: 92.2±24.93 IU/L) was higher than that of HHV-6 DNA negative patients (mean: 78.85±15.13 IU/L). Serum AST level of HHV-6 DNA positive patients (mean: 90.15±24.5 IU/L) was higher than that of HHV-6 DNA negative patients (mean: 72.55± 16.92 IU/L) (Table-7).

Table-7: Detection of ALT& AST activity Levels in HHV-6 DNA patient group

	Positive HHV-6 DNA patients	Negative HHV-6 DNA patients	Statistics	
			T-test	P-value
Mean ALT(IU/L)	92.2±24.93	78.85±15.13	-28.9	0.0012
Mean AST(IU/L)	90.15±24.5	72.55±16.92	-27.21	0.001

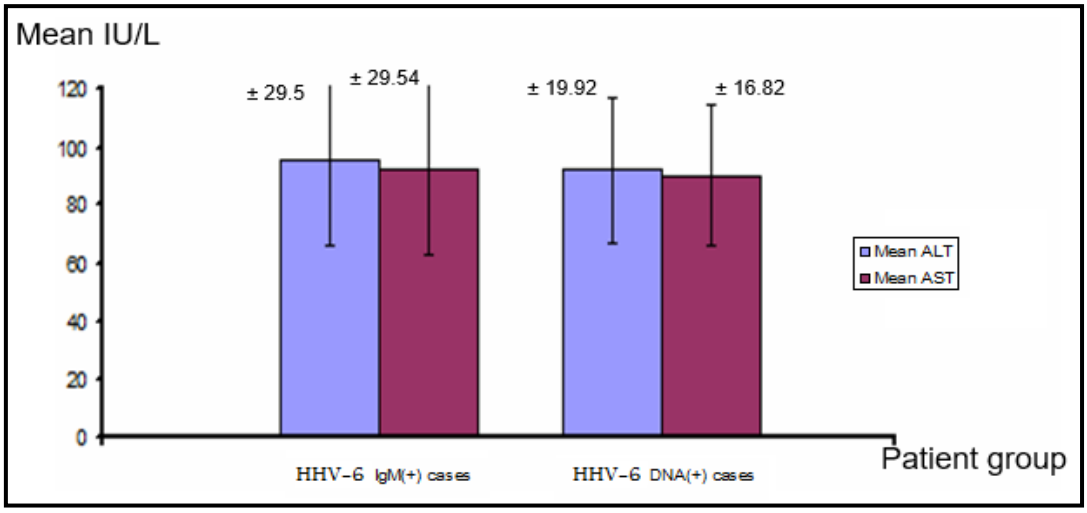

Fig-5: ALT and AST activity levels in patient group with positive HHV-6 IgM and DNA

ALT and AST activity level of Control group (-ve HCV RNA)

In HHV-6 IgM positive group, serum ALT level (mean: 43.8±7.43 IU/L) was higher than that of HHV-6 IgM negative group (mean: 37.23±11.8 IU/L) and also, serum AST level of HHV-6 IgM positive group (mean: 45±4.12 IU/L) was higher than that of HHV-6 IgM negative group (mean: 33.85±11.58 IU/L) (Table-8).

Table-8: Detection of ALT& AST activity levels in HHV-6 IgM Control group

Group / Variable	Positive HHV-6 IgM patients	Negative HHV-6 IgM patients	Statistics	
			T-test	P-value
Mean ALT(IU/L)	43.8±7.43	37.23±11.8	-11.7	0.01
Mean AST(IU/L)	45±4.12	33.85±11.58	-41.1	0.001

In HHV-6 DNA positive group, serum ALT level (mean: 41.71±10.5 IU/L) was higher than that of HHV-6 DNA negative group (mean: 37.3±11.64 IU/L) and also**,** serum AST level of HHV-6 DNA positive group (mean: 43.57±6.65 IU/L) was higher than that of HHV-6 DNA negative group (mean: 33.3±11.63 IU/L) (Table-9).

Table-9: Detection of ALT& AST activity levels in HHV-6 DNA Control group.

Group / Variable	Positive HHV-6 DNA patients	Negative HHV-6 DNA patients	Statistics	
			T-test	P-value
Mean ALT(IU/L)	41.71±10.5	37.3±11.64	11.3	0.1
Mean AST(IU/L)	43.57±6.65	33.3±11.63	4.5	0.4

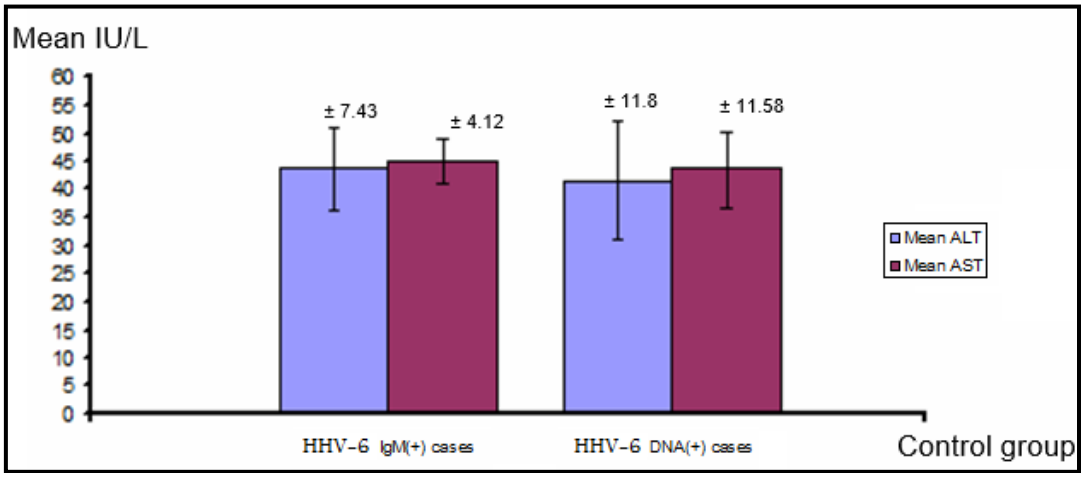

Fig-6: ALT and AST activity levels of control group with positive HHV-6 IgM and DNA

Comparison of Liver enzymes (ALT and AST) between the study groups

In HCV-positive group, serum levels of alanine aminotransferase (ALT) (mean: 92.2± 24.9) in HHV-6 DNA-positive patients was higher than that of HHV-6 DNA-negative patients (mean: 76.84±15.12).Whereas, the serum levels of aspartate aminotransferase (AST) in HHV-6 DNA-positive patients (mean: 90.15±24.54) was higher than that of HHV-6 DNA-negative patients (mean: 72.54 ±16.92) (Table-10).

In HCV-negative group (control group), serum levels of alanine aminotransferase (ALT) (mean: 41.7 ±10.49) in HHV-6 DNA-positive patients was higher than that of HHV-6 DNA-negative patients (mean: 37.29 ±11.6).Whereas, the serum levels of aspartate aminotransferase (AST) (mean: 43.57 ±6.65) in HHV-6 DNA-positive patients was higher than that of HHV-6 DNA-negative patients (mean: 33.3±11.6) (Table-10).

We found that, the activity levels of these enzymes (ALT, AST) were significantly higher in positive group (patient group) than in negative group (control group)

Table-10: Comparison of Liver enzymes (ALT and AST) between two study groups

Groups / Variables		Positive patient group		Control group	
		Positive HHV-6 DNA	Negative HHV-6 DNA	Positive HHV-6 DNA	Negative HHV-6 DNA
Total =84	N	20	33	7	24
	%	23.8%	39.3%	8.3%	28.6%
ALT IU/L	Mean	92.2± 24.9	76.84±15.12	41.7±10.49	37.29±11.6
AST IU/L	Mean	90.15±24.5	72.54±16.92	43.57±6.65	33.3±11.6

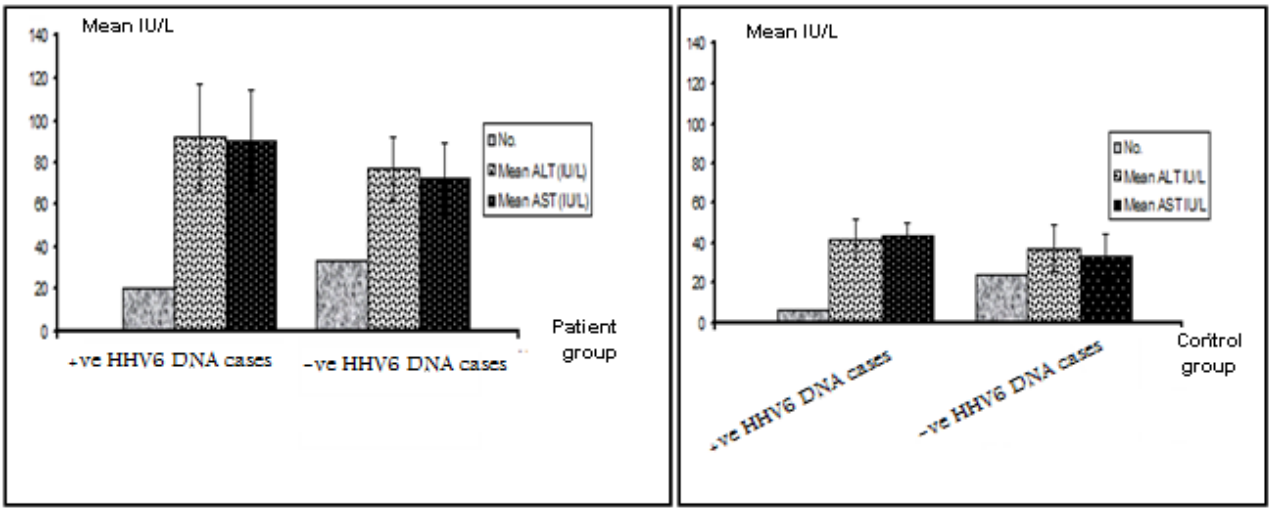

Fig-7: ALT and AST levels among the two study groups

DISCUSSION

Seroprevalence of Human herpesvirus as CMV, EBV and HHV-6 varies in different populations and age groups. Age is one of the risk factors that affect the seroprevalence of both HHV-6-IgM and IgG antibodies [1, 12].

HHV-6-IgM titer level showed an increase until reaching its maximum rate in 32 year old individuals, and HHV-6 IgM seroprevalence increased at age ranged from 30 to 61 years old. Number of cases with positive HHV-6 DNA increased in age ranged from 41 to 61 years old, that may be as a result of herpesvirus reactivation occurs mostly in immunosuppressed persons as well as in elderly because the immune system gets weaker with age [13].

In this study, in HHV-6 DNA positive cases, HHV-6 IgG seroprevalence raised in age ranged from 41 to 61 years old, and in female than in male, while IgM seroprevalence raised in age ranged from 42 to 64 years old. These findings are in complete accordance with those of other studies which emphasize the seroprevalence of HHV-6 not correlated with certain age [13].

The activity levels of ALT and AST enzymes in HCV positive cases, serum activity levels of ALT and AST showed a highly significant ($p<0.001$) elevation in positive HHV-6 IgM than negative cases, also the serum activity levels of ALT and AST enzymes investigated in this study showed a highly significant ($p<0.002$) elevation in positive HHV-6 DNA than negative individuals. These findings indicated to, active

HHV-6 infection in chronic HCV patients had high influence on activity of ALT and AST enzymes by increasing their levels in sera of patients.

The data of this study indicated to, there was statistically significant difference in ALT and AST between patient group with positive HHV-6 IgM and control group with positive HHV-6 IgM ($p<0.001$) also, the data showed significantly difference in ALT and AST activity levels between patient group with positive HHV-6 DNA and control group with positive HHV-6 DNA ($p<0.002$). These data indicated to, the co-infection between HHV-6 and HCV had high effect on liver function.

The results indicated to HHV-6 infection in control group did not cause HHV-6 hepatitis, where HHV-6 hepatitis was evidenced by abnormal (elevation) liver enzyme levels and histopathological changes [8, 14]**.**

Singh *et al*., and Ishikawa *et al*., interpreted that both mean ALT level, and hepatic activity scores were higher in HHV-6-positive patients compared to HHV-6 negative group [15-17].

The data of this study indicated to the infection with HHV-6 was prevalent in HCV patients. Where there may be several reasons for the prevalence of HHV-6 among the HCV patients. Differences in exposure to HHV-6 infection may be excluded because this is ubiquitous virus infecting almost the whole population since infancy [18]. HHV-6 viruses may exert an immunomodulatory effect resulting in enhanced immunosuppression [3, 2]. another factor such as cytokine dysregulation induced by HHV-6 reactivation that could accelerate HCV pathogenesis in critically ill patients.

This hypothesis was supported by the result of this study, that both mean serum ALT and AST levels in HHV-6 positive group were higher than that of HHV-6 negative group in HCV patients. The significant elevation in the serum levels of ALT and AST in HHV-6/HCV patients than in HCV patients and healthy group, reflecting the severity of liver inflammation in HHV-6 infected chronic HCV patients.

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