

## RESEARCH ARTICLE

# E1/E2 of Hepatitis C Virus Genotype-4 and Apoptosis

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### Abstract

Several studies have addressed the possible role of hepatitis C virus genotype-4 (HCV GT4) in apoptosis. However, this still not fully understood. In the current study a re-constructed clone of E1/E2 polyprotein region of the HCV GT4 was transfected into the Huh7 cell line and a human apoptotic PCR array of 84 genes was used to investigate its possible significance for apoptosis. Out of the 84 genes, only 35 showed significant differential expression, 12 genes being up-regulated and 23 down-regulated. The highest-up regulated genes were APAF1 (apoptotic peptidase-activating factor 1), BID (BH3 interacting domain death agonist) and BCL 10 (B-cell CLL/lymphoma protein 10) with fold regulation of 33.2, 30.1 and 18.9, respectively. The most down-regulated were FAS (TNF receptor super family), TNFRSF10B (tumor necrosis factor receptor super-family member 10b) and FADD (FAS-associated death domain) with fold regulation of -30.2, -27.7 and -14.9, respectively. These results suggest that the E1/E2 proteins may be involved in HCV-induced pathogenesis by modulating apoptosis through the induction of the intrinsic apoptosis pathway and disruption of the BCL2 gene family.

**Keywords:** HCV - genotype 4 - apoptosis - caspase - intrinsic pathway - BCL2

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### Introduction

Hepatitis C virus (HCV) infection is one of the major causes of liver complications worldwide (Moradpour and Müllhaupt, 2015). Approximately, 170 million individuals are HCV infected around the world (Clausen et al., 2015). HCV is a member of Hepacivirus group in the family Flaviviridae, with an enveloped glycoprotein. It has a single stranded positive sense RNA genome which encodes a single polyprotein that is cleaved into 10 mature proteins (Revie and Salahuddin, 2011). The lack of any proofreading mechanism is responsible for the high mutation rate of about 10E-5 error/nt (Pavio et al., 2003) and result in about 20-25% difference of the nucleotide sequences, thus HCV isolates have been classified into seven genotypes and several subtypes (Bouchardeau et al., 2007; Di Lorenzo et al., 2011).

Each geographical region is characterized by one of these seven genotypes (Miller and Abu-Raddad, 2010). The cause of approximately 20% of the chronic hepatitis C infection worldwide is HCV genotype-4 (GT4) (Abd Elrazek et al., 2014). The latter is the most common genotype in Egypt, with the highest prevalence of HCV worldwide; of 14-20% HCV infected Egyptians (Abd El-Wahab et al., 2014) and 7 from 1000 persons are affected every year (Okasha et al., 2015). In addition to low treatment response rates in HCV GT4 when compared

to other genotypes (Riad et al., 2015). With the disease rate increase every year, the demand for more research for vaccine development is increasing. The real challenging for vaccine design is extensive world-wide genetic diversity of the virus and rapid viral evolution in infected individuals (Bailey et al., 2015). HCV is enveloped with E1/E2 region that harbors the E1 and E2 viral attachment proteins. E1/E2 role beside viral entry is ill defined up till now (Douam et al., 2015). E1/E2 demonstrates a high level of variability, especially at two sub-regions, HVR1 and HVR2 (Figlerowicz et al., 2010). The genetic variability within this region is mainly responsible for the diversity of HCV genome (Burlone and Budkowska, 2009). The virus non-clearance is associated to the genetic variability, as the highly variable loci in HCV E2 facilitate the virus escape from isolate-specific neutralizing antibodies (Riad et al., 2015) and the resistance to antiviral therapies (Chambers et al., 2005; Binder et al., 2011). We previously addressed genetic divergence in E1/E2 of HCV GT4 in Egypt and a high rate of variability was observed (Hussein et al., 2014).

Yet the mechanisms of viral persistence and HCV associated complication have not been fully explained (Tawar et al., 2015), there are evidences suggesting that liver cell damage in chronic HCV infection is mediated by apoptosis (Bantel et al., 2001). Apoptosis is a vital part of the host anti-viral defense mechanism including HCV infection as it interrupts viral replication and assists in the

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clearance of virus-infected cells but there are increasing data involved apoptosis as a driving force for complication in chronic HCV infection as engulfment of apoptotic hepatocytes by hepatic satellite cells promotes fibrogenesis in damaged liver (Lim et al., 2012). HCV uses a variety of mechanisms that block genes participating in apoptosis, to escape severe immune attacks (Clausen et al., 2015).

A report of different studies with either HCV full-length RNA or cDNA *in vitro* had demonstrated that viral proteins could induce apoptosis (Kalkeri et al., 2001), but it is not recognized which HCV protein affects apoptosis *in vivo* and whether the infectious virions act pro- or anti-apoptotic (Fischer et al., 2007). The apoptotic program is initiated by extrinsic stimuli involving the activation of cell surface receptors such as FAS and the tumor necrosis factor TNF receptor or can be triggered by intrinsic stimuli through the mitochondrial pathway in response to DNA damage, oxidative stress and viral proteins (Fischer et al., 2007; Cerella et al., 2014). Caspases are the key regulatory proteins in both intrinsic and extrinsic pathways (Li and Yuan, 2008). Both apoptotic pathways activate effectors caspases such as caspase 8 and 9, which in turn result in the activation of executor caspase 3, 6 and 7 (Jang et al., 2014). Caspases activation during apoptosis induces morphological and physiological cellular changes, leading to cell death (McIlwain et al., 2015). Apoptosis also involves the modulation of B-cell lymphoma-2 (Bcl-2) family proteins, balancing the anti-apoptotic members with the pro-apoptotic members (Zekri et al., 2011).

It is believed that HCV proteins, especially the envelope proteins, can be toxic to cells independent of direct viral infection by producing the “innocent bystander” effect (Urbaczek et al., 2014). Together, genetic variation of apoptosis related genes is linked with response to interferon-based therapy for HCV infection, and genetic natural clearance of HCV infection (Clausen et al., 2015). Despite these facts, the effect of HCV on apoptosis related expression markers largely remain elusive (Barathan et al., 2015). Very few and controversial studies have the objective assay of the full length of the related sequence and full apoptotic genes. The effects of E2 on apoptosis regulation are more complicated as both pro-apoptotic and anti-apoptotic effects have been observed (Aweya and Tan, 2011). Apoptosis was observed as E2 was transiently expressed in various cell-lines, including Huh7 (Ciccaglione et al., 2004), but in another study, pro-survival activity was expressed in stable cell lines expressing E2 (Zhu et al., 2004). Further studies are needed to understand the contributions of the different HCV genotype 4 proteins to the regulation of apoptosis in liver cells (Barathan et al., 2015). At the present study we aimed to investigate the role of E1/E2 proteins of HCV GT4 on apoptosis *in vitro*, whilst research on the interaction between HCV and cell death pathways will eventually lead to a better understanding of complicated interplay between HCV and its human host and may contribute to the development of antiviral therapeutics and design of efficient strategies for disease control.

## Materials and Methods

### *HCV E1/E2 Construct Synthesis*

According to previously published work on HCV E1/E2 of genotype 4 (Hussein et al., 2014), we chose three different full length HCV E1/E2 sequences of accession numbers JX310299, JX310293 and JX310279. These sequences were assembled from synthetic oligonucleotides by Gene Art® (Thermo Fisher Scientific Technologies, USA). The fragments were cloned into the mammalian expression vector pCMV6-NGFP-CHis\_L305 using SgfI and MluI cloning sites. E1/E2 constructs [Figure 1] and their entry vectors were designed by Origene (Germany). Green fluorescent protein (GFP) used to detect successful expression of constructs. The plasmid DNA was purified from transformed bacteria and its concentration was determined by UV spectroscopy. The final construct was verified by sequencing. The same vector without the insert was used as a transfection control.

### *Culturing and Transfection*

Human Hepatocytes cell line (Huh7) was cultured in DMEM with 10% FBS, 100 units/ml of penicillin, 100 mg/ml streptomycin and 200 mM glutamine.  $4 \times 10^5$  cells were plated in a 6 well plate at the day prior to transfection. 1 µg of HCV E1/E2 constructs and entry vector were transfected into Huh7 cells, using CalPhos™ Mammalian Transfection Kit (Clontech, catalog No 631312). Morphological changes were observed in transfected cells. GFP- positive cells were detected directly using inverted fluorescent microscope with a filter block that excited GFP between 450 and 490 and allowed examination at 520 nm. After 72 hours of culture, supernatants from HCV E1/E2 transfected cells and control were harvested, concentrated and used to infect a new naive Huh7 cells to produce stable cell line expressing HCV E1/E2 and staple cell line expressing entry vector.

### *Apoptosis Assay by Flow Cytometry*

After 3 days of subculture, the both stable cell line expressing HCV E1/E2 and entry vector were harvested, stained, and evaluated for apoptosis by flow cytometry according to the manufacturer's protocol. Briefly, 106 cells were stained with 5 µL of Annexin V-FITC for 20 minutes in the dark, and 10 µL of propidium iodide (5 µg/ml) in 1 × binding buffer was then added to each sample. Next, apoptosis rates were determined by flow cytometry compared to transfected control (FACS Calibur, Becton-Dickinson) using Cell quest software (BD Biosciences, San Jose, CA, USA).

### *RT2 PCR Array Assay*

As an attempt to understand the molecular basis of the apoptotic effect of E1/E2, RT2 Profiler PCR Array was used to analyze the expression level of 84 genes involved in regulation of apoptosis (Human Apoptosis PCR array, PAHS-012Z). Transfected cells were trypsinized, collected and total RNA was extracted using QIAGEN kit (QIAamp RNA Blood mini kit, cat. NO 52304). One µg of RNA was reverse transcribed into cDNA with the use of an RT2 First Strand Kit (SABiosciences), according to the manufacturer's instructions. The resulting cDNA was used as template to perform quantitative PCR analysis

Applied Bio systems 7500 Fast (Thermo FisherScientific, USA).

Relative changes in gene expression were calculated using the comparative threshold cycle (Delta Delta Ct,  $\Delta\Delta Ct$ ) method using the PCR Array Data analysis Web Portal (<http://www.SABiosciences.com/pcr/arrayanalysis.php>). This method first subtracts the Ct (threshold cycle number) of the gene-average Ct of the five housekeeping genes on the array (RPLP0, HPRT1, GAPDH, B2M and ACTB) to normalize for the amount of RNA per sample. Finally the  $\Delta\Delta Ct$  was calculated as the difference between the normalized average Ct of the HCV E1/E2 transfected group and the normalized average Ct of the control group. This  $\Delta\Delta Ct$  value was raised to the power of 2 to calculate the degree of change. All assays were done in triplicates. A p-value of <0.05 was selected to indicate significant differences among groups.

## Results

### Apoptosis Detection with Flow Cytometry Assay

Cells expressing E1/E2 proteins showed reduced cell density and shrinkage when morphologically observed under the microscope; furthermore, green fluorescence confirmed the successful transfection and expression of E1/E2 proteins [Figure 2]. Flow cytometry analysis demonstrated that E1/E2 transfection for 72 h had significant increase in the ratios of early apoptosis cells, while the percentage of viable cells was reduced compared to control cells. Figure 3 shows the three used constructs (1, 2, and 3) with (50.6, 55.5 and 51.5%) early apoptosis rates respectively, in comparison with 23.7% rate in control cells. Since no detectable change was noted in the flow cytometry results between different three constructs,

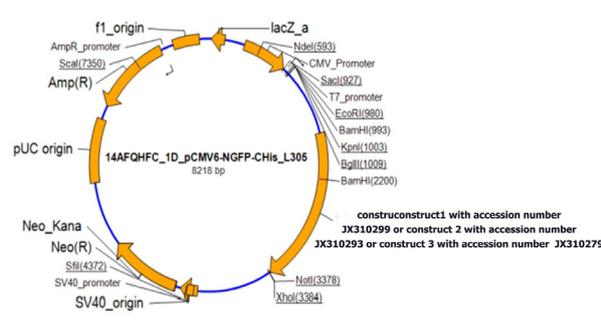


Figure 1. Plasmid Map

Table 1. Differential Up-Regulated expression of Apoptotic Genes after E1/E2 Transfection in HUH7 Cells in Comparison to Control Group

Gene Symbol	Gene description	Fold Regulation	p-value
AIFM1	Apoptosis-inducing factor, mitochondrion-associated, 1	5.1	0.000057
APAF1	Apoptotic protease-activating factor 1	33.2	0.000006
BCL10	B-cell CLL/lymphoma protein 10	18.9	0.000001
BCL2	B-cell CLL/lymphoma protein 2	8.4	0.000001
BCL2A1	BCL2-related protein A1	9.5	0
BCL2L1	BCL2-Like 1	9.4	0.000012
BID	BH3 interacting domain death agonist	30.1	0.000001
BNIP2	BCL2/adenovirus E1B 19 kDa interacting protein 2	8.9	0.00002
CASP4	Caspase-4	16.2	0.000001
CASP7	Caspase-7	5.9	0.000202
CIDEA	Cell death-inducing DFFA-like effector	6.02	0.000004
IL10	Interleukin 10	8.58	0.000012

only one construct was used for the gene expression experiments.

### Gene Expression Results

Out of the 84 studied genes, 35 genes showed significant different patterns of expression in transfected huh7 cell lines in comparison with their corresponding control (Figure 4). Twelve up-regulated genes with fold regulation of 5.1 to 33.2 and 23 down regulated genes with

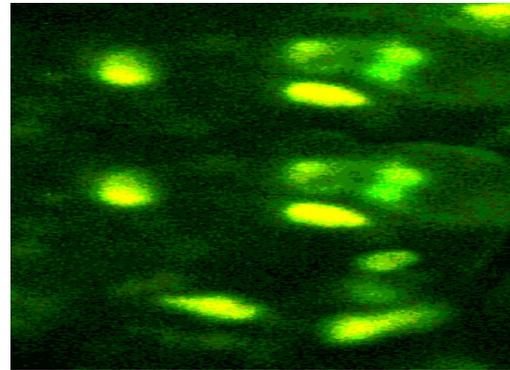


Figure 2. GFP-Positive Huh7 cells Transfected with E1/E2

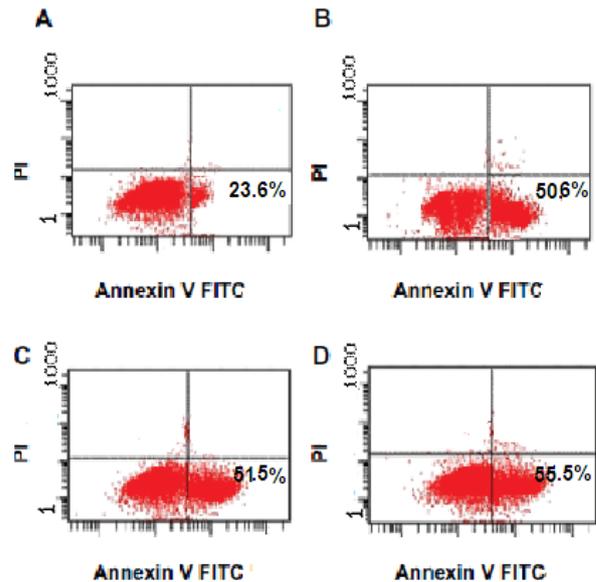
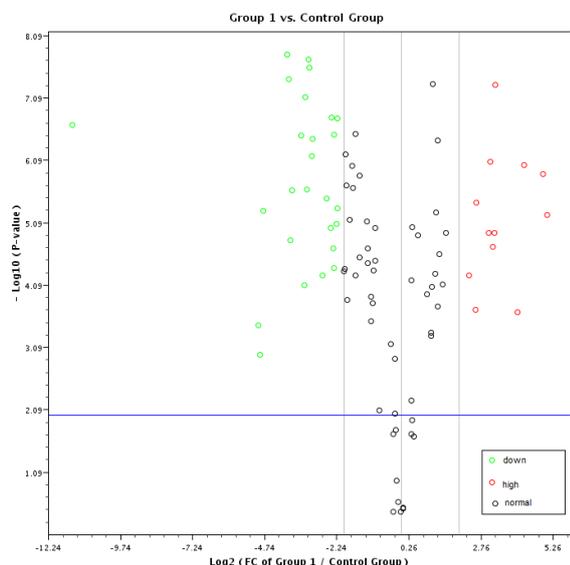


Figure 3. Early Apoptosis Rates of Transfected Cells Measured with flow Cytometry by Treatment with Annexin V-FITC. (A) Cells transfected with entry vector, (B) cells transfected with construct 1, (c) cells transfected with construct 2 and (D) cells transfected with construct 3

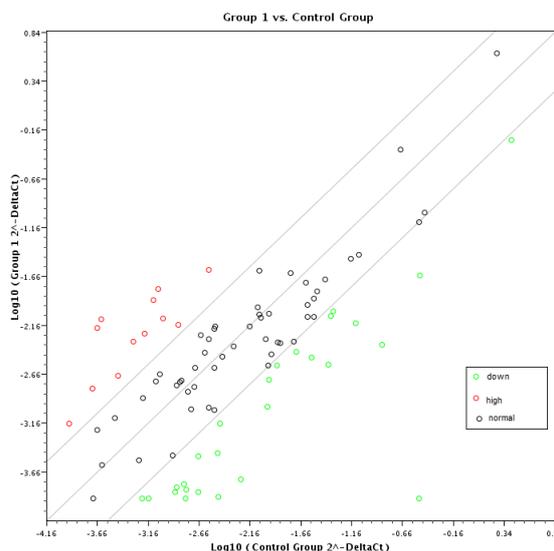
**Table 2. Differential Down Regulated Expression of Apoptotic Genes After E1/E2 Transfection in HUH7 Cells in Comparison to Control Group**

Gene Symbol	Gene description	Fold Regulation	p-value
BAG3	BCL2-associated athanogene 3	-5.5	0.000011
BAK1	BCL2-antagonist/killer 1	-9.6	0.000002
BIK	BCL2-interacting killer (apoptosis-inducing)	-4.8	0.000004
BIRC3	Baculoviral IAP repeat containing 3	-5.3	0
BIRC6	Baculoviral IAP repeat containing 6	-9.2	0
BNIP3L	BCL2/adenovirus E1B 19kDa interacting protein 3-like	-14.7	0
CASP6	Caspase 5, apoptosis-related cysteine peptidase	-10	0
CASP8	Caspase 8, apoptosis-related cysteine peptidase	-8.5	0
CD27	CD27 molecule	-5.1	0
CD40	CD40 molecule, TNF receptor superfamily member 5	-14.6	0.000015
CIDEB	Cell death-inducing DFFA-like effector b	-10.3	0.00008
CRADD	CASP2 and RIPK1 domain containing adaptor with death domain	-13.8	0.000001
CYCS	Cytochrome c, somatic	-6	0
DAPK1	Death-associated protein kinase 1	-8.7	0.000001
FADD	Fas (TNFRSF6)-associated via death domain	-14.9	0
FAS	Fas (TNF receptor superfamily, member 6)	-30.2	0.001103
HRK	Harakiri 9	-5.1	0.000002
MCL1	Myeloid cell leukemia sequence 1 (BCL2-related)	-11.2	0
NOL3	Nucleolar protein 3 (apoptosis repressor with CARD domain)	-9.4	0
PYCARD	PYD and CARD domain containing	-4.7	0.000008
TNFRSF10B	Tumor necrosis factor receptor superfamily, member 10b	-27.7	0.000005
TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10	-6.7	0.000054
TRAF2	TNF receptor-associated factor 2	-5.1	0.000041



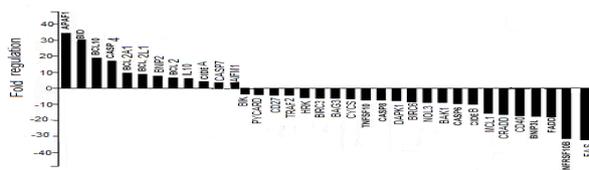
**Figure 4. Volcano Plot Show Differential Expression of 84 Genes in Huh7 Cells Transfected with E1/E2 Construct Versus Huh7 Cells Transfected with Entry Vector.** Twelve genes are significantly up-regulated and 23 genes are significantly down-regulated

fold regulation of -30.2 to -4.7 (Figure 5). The highest up-regulated genes were APAF1 (Apoptotic peptidase-

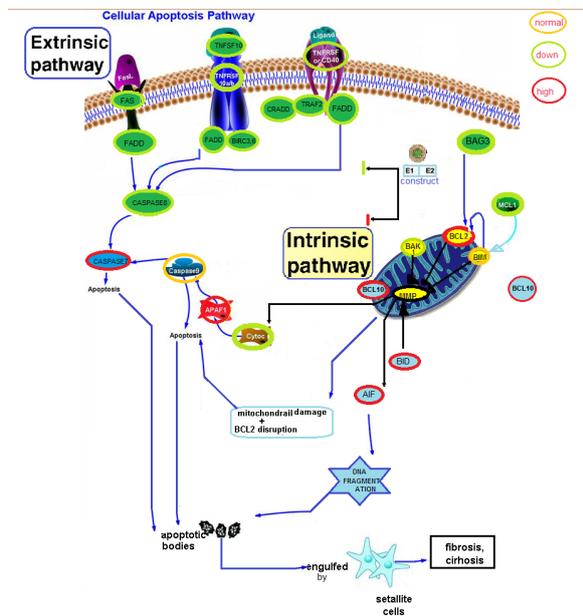


**Figure 5. Scatter plot show fold regulation of 84 genes in Huh7 cells transfected with E1/E2 construct versus control group**

activating factor 1), BID (BH3 interacting domain death agonist) and BCL 10 (B-cell CLL/lymphoma protein 10) with fold regulation of 33.2, 30.1 and 18.9; respectively (Table 1, Figure 6) whereas BCL2A1 (BCL2-related protein A1), CASP4 and BID were the most significant



**Figure 6. Fold Regulation of Up- and Down-Regulated Genes**



**Figure 7. Suggested Cellular Pathway with HCV E1/E2 Involvement in Apoptosis** (The original pathway belongs to SABioscience) **“This image is a modification of QIAGEN’s original, copyrighted image by [zekri et al]. The original image may be found at [http://www.sabiosciences.com/rt\\_pcr\\_product/HTML/PAHS-012Z.html#resource](http://www.sabiosciences.com/rt_pcr_product/HTML/PAHS-012Z.html#resource)”**

up regulated genes of P value 0, 0.000001, 0.000001; respectively. The most down regulated were FAS (TNF receptor super family), TNFRSF10B (tumor necrosis factor Receptor super-family member 10b) and FADD (FAS-associated death domain) with fold regulation of -30.2, -27.7 and -14.9; respectively [Table 2, Figure 6] whereas BNIP3L (BCL2/adenovirus E1B interacting protein 3-like), FADD and CYCS (Cytochrome c, somatic) were the most significant down regulated genes of p value equal 0.00.

## Discussion

HCV pathogenesis is multifactorial phenomena involving both viral and host factors. It has been reported that the high viral envelope proteins mutation rate and apoptosis are involved in pathogenesis and chronicity of hepatitis C (Lee et al., 2005). Recent studies suggested that Hepatocytes apoptosis might also be due to the cytopathic effect of viral proteins (Chiou et al., 2006).

Caspases which are the key regulators in apoptosis grouped on the basis of their known main functions

into two groups, pro-inflammatory and pro-apoptotic groups. Pro-inflammatory caspases (including caspase 4) regulate cytokine maturation during inflammation while pro-apoptotic caspases (such as caspase 7) are known to be mainly involved in mediating cell death signaling transduction. Pro-inflammatory caspases activation can obviously induce apoptosis (Li and Yuan, 2008). Our data showed elevation in expression of caspase 4 and caspase 7. Because of HCV E1/E2 transfection, Caspase 4 induced apoptosis and caspase 7 fragmented DNA as an apoptosis execution protein (Bantel and Schulze-Osthoff, 2003; Wu et al., 2014), driving apoptosis through a caspase-related pathway.

Apoptosis occurs accompanied by a remarkable enhancement of CIDEA (Cell Death-Inducing DFFA-Like Effector A) expression. CIDEA activates apoptosis via DNA fragmentation induction (Lamkanfi and Kanneganti, 2010). CIDEA detectable high expression was shown suggesting the hypothesis of E1/E2 HCV capability to induce apoptosis.

On the other hand, apoptosis process progressed as a consequence of E1/E2 expressions by reduction of apoptosis repressor genes NOL3 (nucleolar protein 3), BIRCE6 (baculoviral IAP repeat containing 6) and BIRC3 (baculoviral IAP repeat containing 30). Apoptosis repressor gene NOL3 inhibits apoptosis and impairs the cellular apoptotic responsiveness to a wide range of stresses and insults (Yonezawa et al., 2011). BIRCE6 and BIRC3 inhibit apoptosis by facilitating the degradation of apoptotic proteins (Ludwig-Galezowska et al., 2011). The NOL3, BIRCE6 and BIRC3 down expression in HCV E1/E2 transfected cells in this study reflected that E1/E2 proteins act as apoptosis inducer that had negative effect on apoptosis repressors by another mechanism.

E1/E2 of HCV GT4 proteins capability to induce apoptosis comes in agreement with studies indicating that E2 expression in culture mammalian cells induces apoptosis (Chiou et al., 2006; Li et al., 2013) furthermore E1 induces apoptosis when E1 baculovirus infected Sf9 insect cells and when it was expressed in Escherichia coli cells (Ciccaglione et al., 2004). Therefore, HCV core protein may not be the only viral protein involved in HCV induction of apoptosis, but E1/E2 proteins play a significant role in apoptosis induction. In contrast, there is a study indicating that transgenic mice expressing HCV core-E1/E2 proteins developed significantly larger tumors than transgenic mice expressing HCV core proteins alone or non-transgenic mice (Kamegaya et al., 2005). This study indicates that E1/E2 expression affect apoptosis blocking. Another study showed that apoptosis was inhibited in Huh7 cells expressing HCV E2 (Lee et al., 2005). Additionally, it is regarded that this study was the first demonstration of HCV GT4 E1/E2 contribution in apoptosis-related gene interaction.

Apaf-1 is a key gene in the apoptotic signaling cascade (Shalaeva et al., 2015). Apaf-1 mediates activation of Apaf-3 (Apoptotic protease-activating factor 3), leading to the activation of caspase-3, so apoptosis process proceed. It was expressed with high significant values in HCV E1/E2 transfected cells compared to control cells. Increasing of Apaf-1 level gave a clear picture on how

apoptosis pathways proceed; this is because Apaf-1 is involved in the intrinsic (mitochondrial) pathway (Tanase et al., 2015). Furthermore, BID, IL-10 and AIFM1 which participate in the intrinsic pathway were highly expressed in transfected group in response to E1/E2 expression activities, where BID regulates the permeabilization of the outer mitochondrial membrane (OMM) by its pro-apoptotic activity (Manara et al., 2009). IL-10 (Interleukin 10) serves as a potent inducer of the intrinsic, mitochondrial apoptosis (Bailey et al., 2006). AIFM1 (Apoptosis inducing factor, mitochondrion-associated, 1) is released in response to apoptotic stimuli from the mitochondrion inter-membrane space into the cytosol and to the nucleus, where it acts as a pro-apoptotic factor (Hussain and Garantziotis, 2013). Therefore we suggest that E1/E2 have induced apoptosis in a mitochondrial intrinsic pathway supporting the previous observation that HCV E2 may induce apoptosis of Huh-7 cells via a mitochondrial-related caspase pathway and sustain the hypothesis that viral induction of apoptosis is mainly by intrinsic pathway.

Extrinsic pathway of apoptosis begins outside the cell through activation of pro-apoptotic receptors on the cell surface which are members of the tumor necrosis factor receptor gene super family (TNF) (Hildebrandt et al., 2015). TNF family recruits FADD that activates caspase-8, which directly activates the apoptosis executioner caspases. Some members of TNF family which are TNFRSF10B, TNFSF10 (Tumor Necrosis Factor (Ligand) Super family, Member 10), CD27 molecule, CD40 molecule, HRK (Harakiri, BCL2 interacting protein (contains only BH3 domain) and TRAF2 (TNF receptor-associated factor 2) (Hase et al., 2002; Lin and Richburg, 2014) were down regulated. Furthermore FAS, FADD and caspase-8 which are key players of the extrinsic pathway (Chaabane et al., 2014) were also down regulated. CRADD (Casp2 and RIPK1 domain containing adaptor with death domain) role in the extrinsic pathway appears in recruiting caspase-2 that plays a central role in execution-phase of cell apoptosis (Ahmad et al., 1997). CRADD expression was down regulated in our results. Here, we propose that HCV E1/E2 expression had activated the intrinsic apoptosis pathway through blocking the extrinsic pathway.

BCL2 family is the best regulator for apoptosis (Tsujimoto, 1998). Alteration in BCL2 gene family members because of viral protein interaction interrupts normal pathway of apoptosis (Alibek et al., 2014). The results of the present study showed that E1/E2 had disrupted BCL2 family members' expression through both; down regulation of pro-apoptotic genes as Bak1 (BCL2-Antagonist Killer 1), BIK (BCL2-like 10) and BNIP3L and up regulation of anti-apoptotic genes as Bcl-2 (B-cell CLL/lymphoma protein 2), BCL2A1, BCL2L1 (BCL2-Like 1), and BNIP2 (BCL2/adenovirus E1B 19 kDa interacting protein 2) (Jin et al., 2012; Zhang et al., 2015). This disruption in expression of BCL2 gene family members may be the driving force for chronic HCV related apoptosis pushing the way for viral persistence and explaining disease complications.

Moreover, CYCS which was down regulated was shown to be related to disruption in BCL2 family. Since

CYCS plays a role in apoptosis through activation of pro-apoptotic members of BCL2 family (Skulachev, 1998). CYCS was down regulated; also some pro-apoptotic members were down regulated.

In the present study BCL10 (B-cell CLL/lymphoma protein 10) which supports apoptosis and interacts with the other apoptosis regulators as TRAF2 and apoptosis inhibitor proteins (Yui et al., 2001) was highly expressed. In contrast, TRAF2 and apoptosis inhibitors were down regulated. BAG3 (BCL2 Associated athangogene 3) and MCL1 (Myeloid cell leukemia sequence 1) which act as anti-apoptotic genes in BCL2 family (Vogler et al., 2009; Jin et al., 2012) were down expressed in transfected cells. BCL2 family genes, BCL10, BAG3 and MCL1 data confirmed that E1/E2 had the capability of inducing apoptosis and disrupting BCL2 family members' expression.

In summary, our findings have revealed that E1/E2 proteins may be involved in HCV-induced pathogenesis by modulating apoptosis. Modulation of apoptosis was performed through the induction of intrinsic apoptosis pathway accompanied with disruption in BCL2 genes family. Therefore, we suggest that HCV GT4 E1/E2 may have a role in interruption of apoptosis by dis-regulating its process leading virus persistence to cause liver damage.

The present study suggested not only investigating the molecular basis of E1/E2 function in apoptosis but also working on the protein level in the future studies. This leads to more understanding of persistent HCV infection, tumorigenesis and pave the way for establishing effective E1/E2 vaccine.

List of Abbreviations: HCV: hepatitis C virus; GT4:HCV genotype 4; E1/E2:Hepatitis C virus (HCV) envelope protein;AIFM1s: Apoptosis inducing factor, mitochondrion-associated, 1; APAF1:Apoptotic peptidase-activating factor 1; BCL10: B-cell CLL/lymphoma protein 10; BCL2: B-cell CLL/lymphoma protein 2; BCL2A1: BCL2-related protein A1; BCL2L1: BCL2-Like 1; BID: BH3 interacting domain death agonist; BNIP2: BCL2/adenovirus E1B 19 kDa interacting protein 2; CASP4: CASP4; CASP7: CASP7; CIDEA: Cell Death-Inducing DFFA-Like Effector A; IL10: Interleukin 10; BAG3: BCL2 Associated athangogene 3; BAK1: BCL2-Antagonist Killer 1; BIK: BCL2-like 10; BIRC3: baculoviral IAP repeat containing 3; BIRC6: Baculoviral IAP repeat containing 6; BNIP3L: BCL2/adenovirus E1B interacting protein 3-like; CASP6: CASP6; CASP8: CASP8; CD27: CD27 molecule; CD40: CD40 molecule; CIDEB: Cell Death Inducing DFFA-like effector b; CRADD: Casp2 and RIPK1 domain containing adaptor with death domain; CYCS: Cytochrome c, somatic; DAPK1: death-associated protein kinase 1; FADD: FAS-associated death domain; FAS: TNF receptor super family; HRK: Harakiri, BCL2 interacting protein (contains only BH3 domain); MCL1: Myeloid cell leukemia sequence 1 (BCL2-related); NOL3: Nucleolar protein 3 (apoptosis repressor with CARD domain); PYCARD: PYD and CARD containing domain; TNFRSF10B: tumor necrosis factor Receptor superfamily member 10b; TNFSF10: Tumor Necrosis Factor (Ligand) Superfamily, Member 10; TRAF2: TNF receptor-associated factor 2; OMM: the

outer mitochondrial membrane.

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