DETECTION OF CIRCULATING HEPATITIS C VIRUS NON-STRUCTURAL PROTEIN IN CHRONIC HEPATITIS C VIRUS AND IN HAEMODIALYSIS PATIENTS

Ahmed Barakat Barakat [(1)], Hossam El-Din Ahmed Ghanem [(1)], Rashed Ibrahem Rashed Al Nagdy[(1)], Abdelfattah M. Attallah[(2)],

[(1)]Microbiology department, Faculty of Science, Ain shams University, Cairo, Egypt
[(2)]Biotechnology Research Center, New Damietta, Egypt

ABSTRACT

Infection with hepatitis C virus (HCV) is a major cause of chronic liver disease. On the other hand, Nosocomial transmission of HCV is a concern in haemodialysis (HD) units worldwide. In these patients, blood transfusions and long term dialysis are risk factors for transmission of HCV. Diagnosis of HCV infection is currently based on the detection of anti HCV antibodies by ELISA, and is confirmed by HCV RNA. The aim of the present study was to identify and detect the circulating non structural protein by using ELISA ,SDS-PAGE and Western blot techniques and to evaluate the usefulness of the detection of HCV antigen using ELISA for therapeutic follow-up (at 0 times, 12, 24 and 48 weeks) in selected 10 positive HCV-RNA patients at 0 times . Serum samples of 75 chronic hepatitis C (CHC) patients, serum samples of 75 haemodialysis patients and 25 healthy individual's sera as a negative control were included in this study. HCV antigen was detected in these samples using ELISA and Western blotting techniques. Western blot analysis showing a single immune reactive band in serum of CHC and haemodialysis patients infected with HCV at 27-KDa. ELISA technique was applied to detect the 27-KDa antigen. The cutoff level of ELISA above or below which the tested sera were considered positive or negative was calculated and was found to be 0.28 Based on this cut off the HCV antigen was detected in 80 % of CHC patients and in 36 % of haemodialysis patients. While, it was found that all healthy individuals used as a control were 100 % negative for HCV antigen. Furthermore, detection of HCV-RNA using nested PCR and HCV-NS4 antigen using ELISA for pre-therapeutic and therapeutic (combined interferon (IFN) and ribavirin therapy) at 0 time, 12, 24 and 48 weeks in 10 HCV-infected persons undergoing treatment was studied. The detection of HCV-NS4 antigen for these 10 positive HCV-RNA samples at (12 weeks and 24 weeks) showed that (60 %) were responsive for treatment and it was found that (40 %) did not respond.

Key words: HCV, diagnosis, non-structural protein, detection, haemodialysis
Running Title: HCV-NS4 Antigen in patients with haemodialysis.

INTRODUCTION:

The global public health impact of chronic HCV infection and consequent liver disease continues to grow in numbers. It has been estimated that there are over 170 million carriers of HCV worldwide with increasing incidence of new infections **(Dennis *et al.*, 2005)**. HCV is a major health problem in Egypt **(Ray *et al.*, 2000)**. It has the largest epidemic of hepatitis C virus in the world. The recently demographic health survey (EDHS) said that about 14.7 % of 78 million is infected and about 10 % of them still infectious to other people. The issue of treatment for those that develop HCV related liver disease is essentially medical care crisis for the country **(www.Hawaii.com)**. On the other hand, HCV is a significant problem in the management of haemodialysis patients. A high prevalence of

HCV infection in haemodialysis patients in addition to the risk factors such as the number of blood transfusions or duration on haemodialysis have been identified **(Hinrichsen *et al.*, 2002)**. HCV is a prevalent infectious disease generally contracted via blood and blood products infected with HCV **(Jimenez *et al.*, 2009).** The risk for HCV infection is dramatically increased during transfusion. Most of patients will progress to chronic hepatitis and some will even develop cirrhosis and hepatocellular carcinoma **(Fung *et al.*, 2009).**

Due to the lack of efficient prevention, such as theraby and vaccines, an accurate early diagnosis is essential for the preventing transmission of the disease **(Xie *et al.*, 2007).** Routine anti HCV antibody detection is not applicable to confirm viral infection in the early phase of HCV infection before anti -HCV antibody has been produced **(Re *et al.*, 2005).** In addition, the HCV- RNA assay is difficult and lacks reproducibility. The high cost of nucleic acid amplification testing (NAT) seemed to preclude its use in developing countries such as Egypt **(Gallarda and Dragon 2000; Fabrizi *et al.*, 2005; Tobler *et al.*, 2005).** Although HCV-RNA detection is the only systems for pre-therapeutic and therapeutic (combined interferon (IFN) and ribavirin therapy) follow-up of HCV-infected persons undergoing treatment. Since RNA detection has many disadvantages such as labor, intensive and very expensive, so many laboratories have sought to replace HCV-RNA detection by other HCV markers. So our aim from this study is to employ cheap techniques which offer an alternative to NAT. Such techniques like ELISA, SDS-PAGE and Western blot assays which can be applied to detect the circulating HCV non structural protein in the tested sera and to evaluate the usefulness of the detection of HCV antigen using ELISA for therapeutic follow-up (at 0 times, 12, 24 and 48 weeks) in selected 10 positive HCV-RNA patients at 0 times.

MATERIALS AND METHODS:

1. Samples collection:

One hundred seventy five serum samples were collected from Cairo University Hospitals, Cairo, Egypt. They were classified into three groups: Group (I): Serum samples of 25 healthy individuals were used as negative control, they included 19 males and 6 females; aged from 22 to 63 years (mean age of 42.5 years). They were negative for anti-HCV antibodies and they had normal liver and kidney functions tests. Group (II): Serum samples were collected from 75 haemodialysis patients. They included 51 males and 24 females; aged from 24 to 68 years (mean age of 46 years). Information on the age of patients, duration of haemodialysis, number of blood transfusions and renal diagnosis was obtained from patient records and interviews. Group (III): Serum samples were collected from 75 chronic hepatitis C patients (CHC). They included 56 males and 19 females; aged from 25 to 59 years (mean age of 42 years). They were positive for anti-HCV antibodies testing. Serum were separated from blood samples and stored at -20°C. An informed consent was obtained from all individuals participating in the present study, and they were fully informed concerning the nature of the disease and the diagnostic procedures involved.

2. Protein content determination:

Protein content of the antigenic solutions was measured colorimetrically using the method of **(Lowry *et al.*, 1951)**. The antigenic solutions (20 µl) were added separately per 100 µl of working solution and serial concentrations of bovine serum albumin (BSA) standard protein was tested in parallel. Samples were mixed well using a vortex mixer and allowed to stand at room temperature for 10 min. Aliquots of 10 µl of 1N Folin and Ciocalteu's reagent were added to each tube then the contents were mixed well using the

vortex and allowed to stand at room temperature for 30 minutes. A blue color was developed and the absorbance value was read at 490 nm using ELISA reader.

3. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE):

Serum samples at 25µg/lane were separated by SDS-PAGE according to the method of Laemmli **(Laemmli, 1970).** The solution of proteins (antigens) to be analyzed is first mixed with SDS, an anionic detergent which denatures secondary and non disulfide linked tertiary structures, and applies a negative charge to each antigen in proportion to its mass. Serum samples from haemodialysis either infected with HCV or not, chronic hepatitis C patients and healthy individuals were analyzed by 12 % one-dimensional SDS-PAGE under reducing conditions. A mixture of reference proteins (Sigma Chemical Co., St. Louis, MO, USA) was run in parallel which includes Myosin (215.0 kDa), phosphorylase B, (120.0 kDa), Bovine serum albumin (84.0 kDa), Ovalbumin (60.0 kDa), carbonic anhydrase (39.2 kDa), trypsin inhibitor (28.0 kDa), and lysozyme (18.3 kDa). Then gel was stained with Coomassie blue. The Coomassie blue stained separated polypeptides have a wide range of molecular weights ranged from 215 KDa to 18.3 KDa.

4. Immunobloting technique (Western blot):

Serum samples separated by SDS-PAGE were transferred from the polyacrylamide gel to nitrocellulose (NC) sheet according to the method of Towbin **(Towbin *et al.*, 1979).** NC filter was blocked in blocking buffer and was then rinsed in TBS and incubated with anti HCV-NS4 mono-specific antibody with constant shaking overnight then washed in TBS three times, 10min each. The NC filter was incubated with goat anti- rabbit IgG alkaline phosphatase conjugate, for 2 hours with dilution of 1: 500 followed by washing in TBS as mentioned before. The target antigen for anti HCV-NS4 mono-specific antibody was visualized by incubating the NC filter in substrate solution (BCIP/NBT) system. Then the reaction was stopped by distilled water.

5. Detection of HCV antigen using ELISA:

According to **Attallah *et al.*, (2008)** Diluted serum sample (1:250), in coating buffer (pH 9.6), was tested (50 µl per well) for HCV antigen. In brief, coated ELISA plate was sealed with an acetate plate sealer and incubated overnight at 2-8 ºC. After blocking of free binding sites, specific anti-HCV NS4 antibody in PBS-T20 was added (50 µl per well) and incubated at 37 ºC for 2 h. After washing, 50 µl/ well of anti-rabbit IgG alkaline phosphatase conjugate diluted in 0.2% (w/v) BSA in PBS-T20, was added and incubated at 37 °C for 1 hr. The amount of coupled conjugate was determined by incubation with p-nitrophenyl phosphate substrate for 30 min at 37 °C. The reaction stopped and absorbance was read at 490 nm using ELISA reader (Ó960 Metretech, Germany).

6. Qualitative polymerase chain reaction (PCR) for detection of hepatitis C virus in serum samples:

The extraction of HCV-RNA carried out using simple, rapid and reliable protocol for the small-scale purification of DNA and RNA from human serum according to **Boom *et al.*, (1990).** Nested PCR was carried out using one primer set from 5-UTR.Then analysis of the amplified PCR products by using agarose gel electrophoresis and DNA marker. Agarose gel electrophoresis of DNA was performed according to **Sambrook *et al.*, (1989).** Then the gel was immersed in water containing ethidium bromide (10mg/ml) for 30 minutes at room temperature. Excess of ethidium bromide was removed by washing the gel in 1X TAE for 30 minutes. Gel were examined by UV light on a UV transilluminator (FBTIV 816-

transilluminator), and photographed using a Polaroid camera (Fisher Biotech Electrophoresis Systems Photo-Documentation Hood FB-PDH 1414) with Polaroid 667 film.

7. Statistical Analysis:

All statistical analyses were done by a statistical software package (SPSS 15.0 for Microsoft Windows, SPSS Inc.). Descriptive results were expressed as mean ± SD and range or number (percentage) of patients with a condition. Differences in continuous variables were assessed using student t-test or ANOVA and X2 test for categorical variables. All tests were two-tailed and statistical significance assessed at the 0.05 level.

RESULTS

1. Protein content determination:

A standard calibration curve was plotted using serial concentration of the standard BSA protein. The unknown concentrations of the antigenic solutions were determined from the curve, **(fig. 1)**.

BSA Concentration (mg/ml)	0	0.25	0.5	1	1.5	2	2.5	3
Absorbance at 490 nm	0	0.05	0.09	0.18	0.24	0.32	0.37	0.42

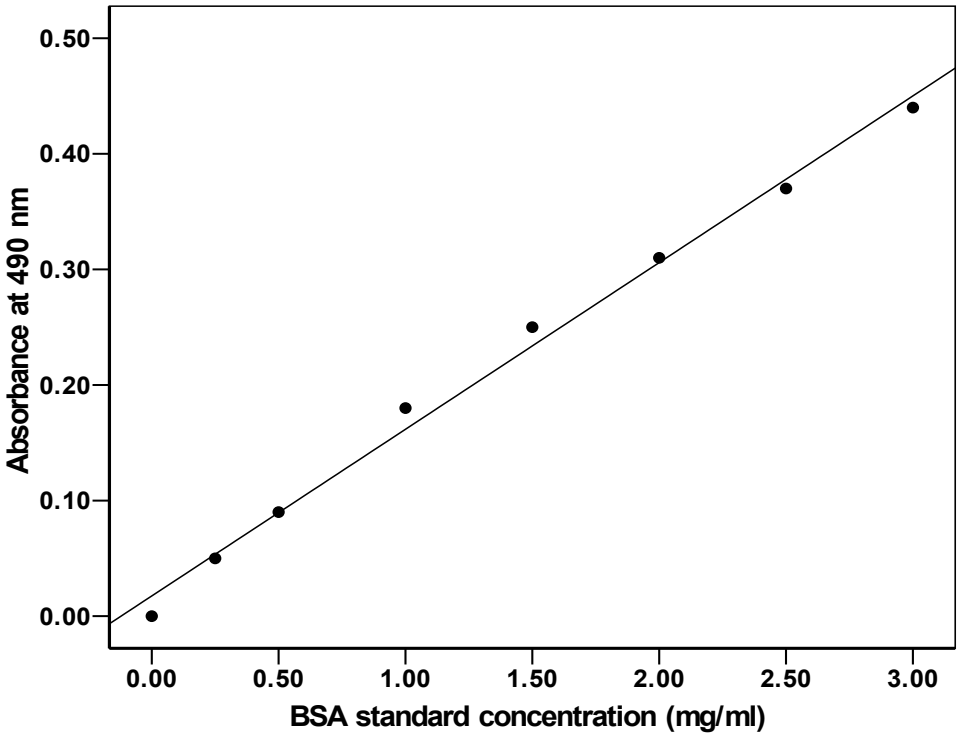

Fig. (1): Standard calibration curve of bovine serum albumin

2. SDS-PAGE of serum samples from chronic HCV patients, haemodialysis HCV infected or not infected patients and healthy individuals:

The resolved bands were identified in serum samples after staining the gel by Coomassie brilliant blue R-250 dye **(Fig.2).**

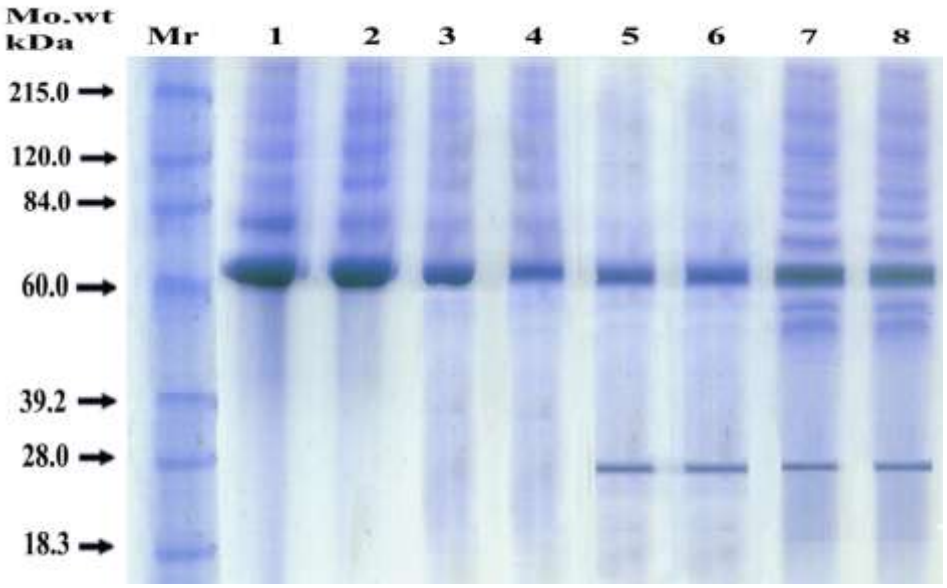

Fig.(2): Coomassie blue stained SDS-PAGE showing the polypeptide pattern of serum samples from haemodialysis and chronic hepatitis C patients and healthy individuals. **Lanes (1-2):** serum samples from healthy individuals. **Lanes (3-4):** serum samples from haemodialysis patients non-infected with HCV. **Lanes (5-6):** serum samples from haemodialysis patients infected with HCV. **Lanes (7-8):** serum samples from chronic hepatitis C patients.

Molecular weight marker (Mr.) A mixture of reference proteins (Sigma Chemical Co., St. Louis, MO, USA) .

3. Immunoblotting of HCV antigen (HCV-NS4) in serum samples by polycolonal antibody:

The results in **Fig (3)** show that an intense sharp band was shown in samples from haemodialysis patients infected with HCV as well as chronic hepatitis C patients at 27 KDa. Results also show no reaction was detected in serum samples from healthy individuals and from haemodialysis patients non-infected with HCV**.**

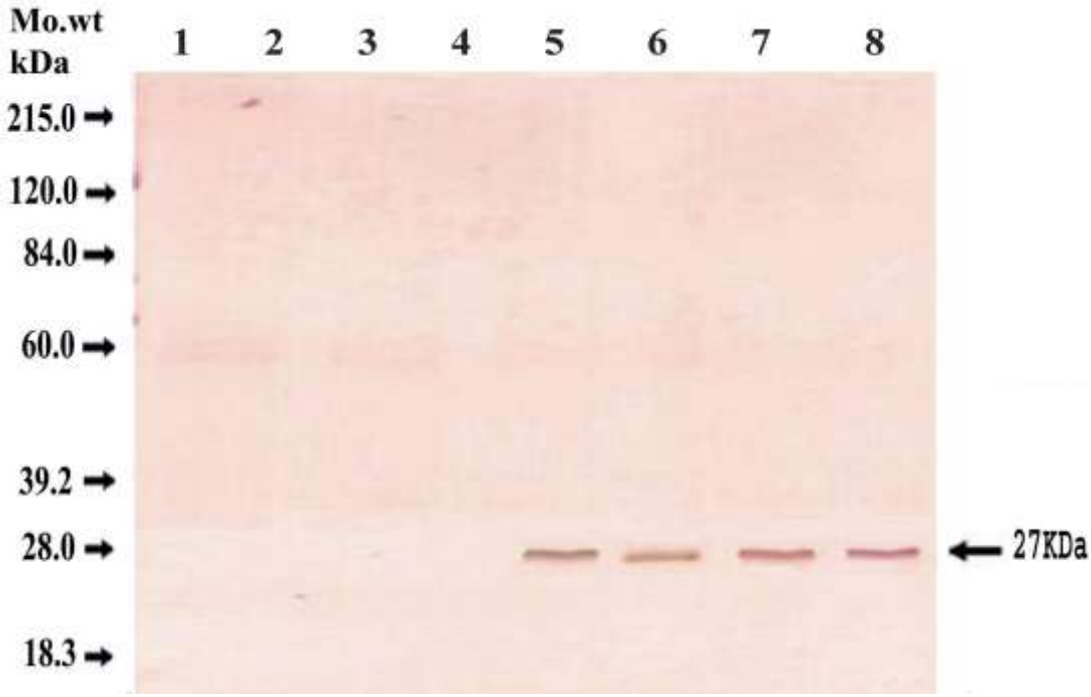

Fig.(3): Immunoblots of Anti HCV-NS4 antibody on serum samples from haemodialysis and chronic hepatitis C patients and healthy individuals. **Lanes (1-2):** serum samples from healthy individuals. **Lanes (3-4):** serum samples from haemodialysis patients non-infected with HCV. **Lanes (5-6):** serum samples from haemodialysis patients infected with HCV. **(7-8):** serum samples from chronic hepatitis C patients. **Standard molecular weight marker:** A mixture of reference proteins (Sigma Chemical Co., St. Louis, MO, USA) ranging from 18.3 to 215 KDa.

4. Detection of HCV antigen in serum using ELISA:

The expression of HCV antigen in serum samples was detected by using the ELISA technique. The cut-off level of ELISA above or below which the tested sample is considered positive or negative was calculated as the mean ELISA optical densities from 16 individuals non-infected with HCV ± 3 standard deviation**.** In addition we used serum samples from 8 chronic hepatitis C patients and serum samples from 8 haemodialysis patients infected with HCV as positive control. The cut-off level was set at 0.28 **(Fig. 4).**

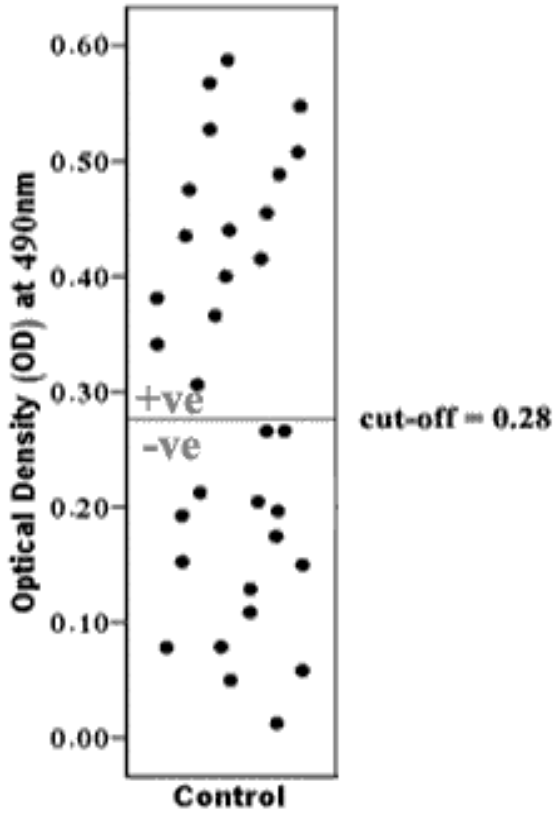

Fig.(4): Cut-off level of HCV antigen in sera of haemodialysis and chronic hepatitis C patients using ELISA

5. Detection rate of HCV antigen (HCV-NS4) in sera of chronic hepatitis C and haemodialysis patients using ELISA:

An interesting result was shown in **Fig.(5)** where the HCV antigen was detected in 80 % of chronic hepatitis C patients. The remaining 20 % were negative for HCV antigen using ELISA technique**.** The detection rate of HCV antigen was very obvious in chronic hepatitis C patients (80%) comparing with those of healthy individuals. Also The results revealed that the detection rate of HCV antigen in 75 haemodialysis patients was shown to be 36 % .Whereas 64 % were negative for HCV antigen using ELISA technique. Furthermore, it was found that all healthy individuals used, in this study, as a control were 100 % negative for HCV antigen. Moreover; there was a statistical significant difference in the distribution of HCV-NS4 antigen between the three groups ($X^2 = 74.00$, $P < 0.0001$).

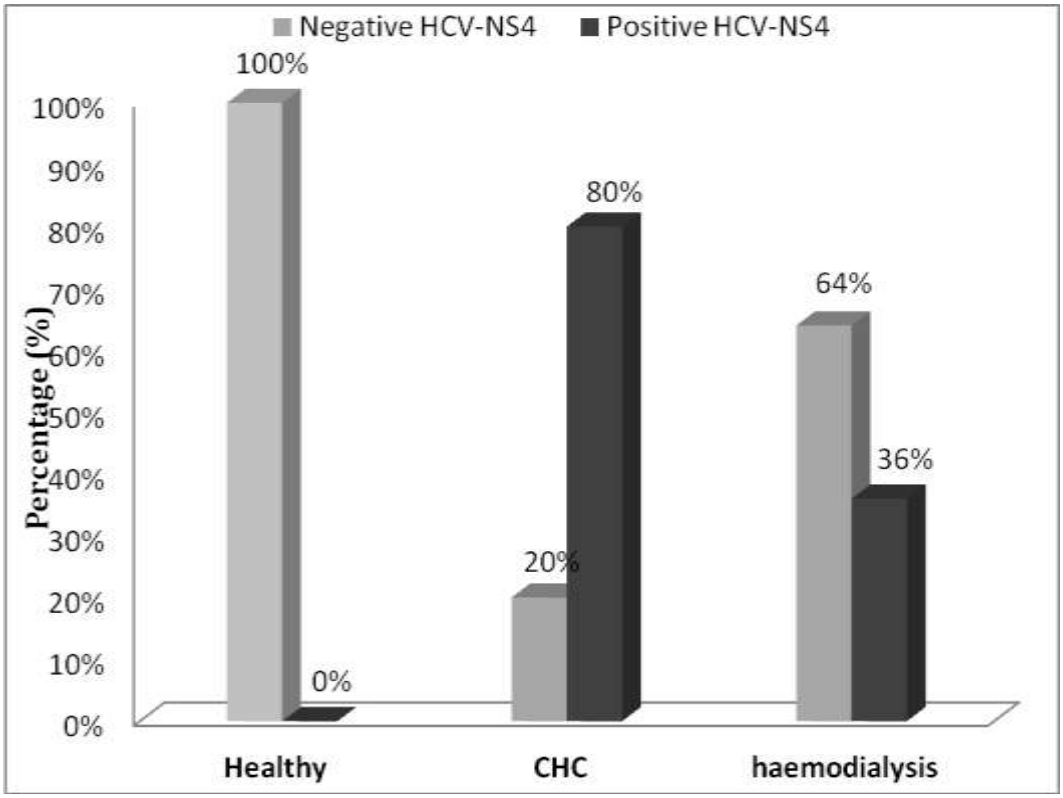

Fig. (5): Detection rate of HCV-NS4 antigen in sera of CHC and haemodialysis patients in comparison with healthy individuals using ELISA**.**

6. Detection of HCV-RNA in chronic hepatitis C patients using nested PCR

The nested polymerase chain reaction (PCR) using amplification of reversibly transcribed RNA is considered the gold standard for the detection of the viral nucleic acid in serum and tissue samples and is thought to indicate active infection with the HCV. The nested PCR was performed to detect the HCV- RNA in serum samples. First, the viral RNA in serum was extracted and then, nested PCR was carried out using one primer set from 5-UTR which includes external primers: [Sense (5′CCA TGG CGT TAG TAT GAG TG 3′) and Antisense (5′ TGC TCA TGG TGC ACG GTC TA 3′)] and internal primers: [Sense (5′ AGA GCC ATA GTG GTC TGC GG 3′) and Antisense (5′ CTT TCG CGA CCC AAC ACT AC 3′)]. The size of the amplified PCR products was determined using agarose gel electrophoresis and DNA marker. The positive HCV-RNA cases showing sharp band at 150 bp (**Fig.6).**

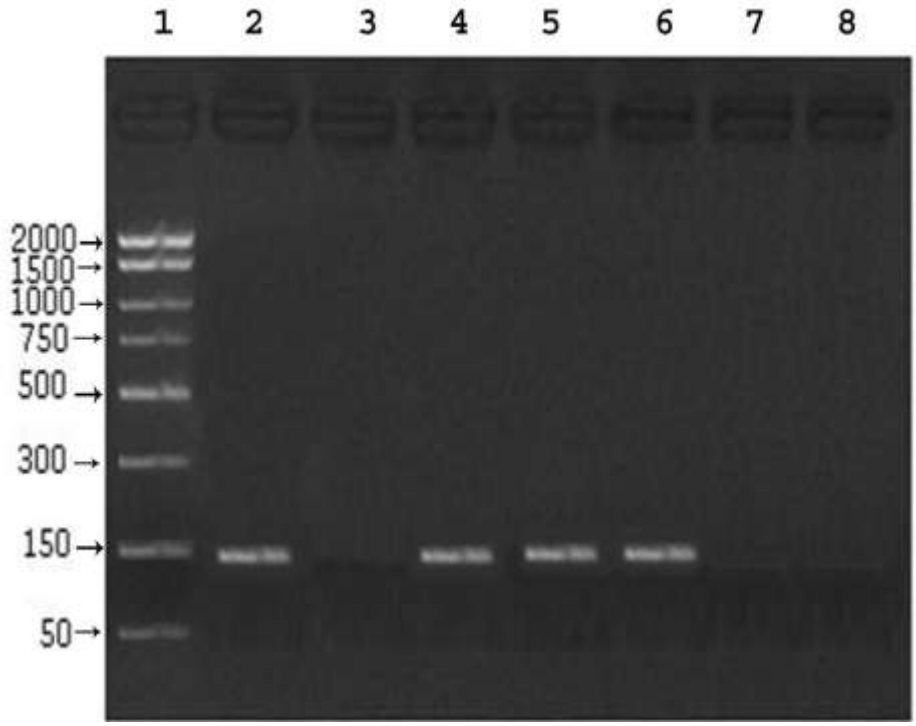

Fig. (6): Agarose gel electrophoresis of PCR products for HCV-RNA.

Lane 1: DNA ladder
Lane 2: Positive control
Lane 3: Negative control.
Lanes 4-6: Serum samples from CHC patients.
Lanes 7-8: Serum samples of individuals non-infected with HCV.

7. Relation between level of HCV antigen and treatment in chronic hepatitis C patients:

Detection of HCV-RNA using nested PCR and HCV-NS4 antigen using ELISA for pre-therapeutic (0 time) and post therapeutic (combined interferon (IFN) and ribavirin therapy) at 12, 24 and 48 weeks for 10 HCV-infected persons undergoing treatment were summarized in **table (1)**. The detection of HCV-NS4 antigen for these 10 positive HCV-RNA samples at 12 weeks and 24 weeks showed that (60 %) were responsive for treatment and it was found that (40 %) did not respond. **(Fig. 7).**

Table (1): Detection of HCV-RNA using nested PCR and HCV antigen using ELISA for pre-therapeutic and therapeutic (combined interferon (IFN) and ribavirin therapy) at 0 time, 12, 24 and 48 weeks in HCV-infected persons undergoing treatment.

Samples	Follow up of treatment							
	0 time		12 weeks		24 weeks		48 weeks	
	HCV RNA[a]	HCV Ag[b]	HCV RNA	HCV Ag	HCV RNA	HCV Ag	HCV RNA	HCV Ag
1	+Ve	+Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
2	+Ve	+Ve	Wk +Ve[c]	Wk +Ve	-Ve	-Ve	-Ve	-Ve
3	+Ve	+Ve	Wk +Ve	Wk +Ve	-Ve	-Ve	-Ve	-Ve
4	+Ve	+Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
5	+Ve	+Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
6	+Ve	+Ve	Wk +Ve	Wk +Ve	-Ve	-Ve	-Ve	-Ve
7	+Ve	+Ve	+Ve	+Ve	N.T.[d]	N.T.	N.T.	N.T.
8	+Ve	+Ve	+Ve	+Ve	N.T.	N.T.	N.T.	N.T.
9	+Ve	+Ve	+Ve	+Ve	N.T.	N.T.	N.T.	N.T.
10	+Ve	+Ve	+Ve	+Ve	N.T.	N.T.	N.T.	N.T.
Negative	0/10	0/10	3/10	3/10	6/10	6/10	6/10	6/10
Positive	10/10	10/10	7/10	7/10	4/10 N.T.[d]	4/10 N.T.[d]	4/10 N.T.[d]	4/10 N.T.[d]

HCV RNA[a]: HCV-RNA using nested PCR (qualitative).
HCV Ag[b]: HCV-NS4 antigen using ELISA.
Wk +Ve[c]: week positive
N.T.[d]: No treatment for these patients (treatment was stopped for non-responder after 12 or 24 weeks of treatment).

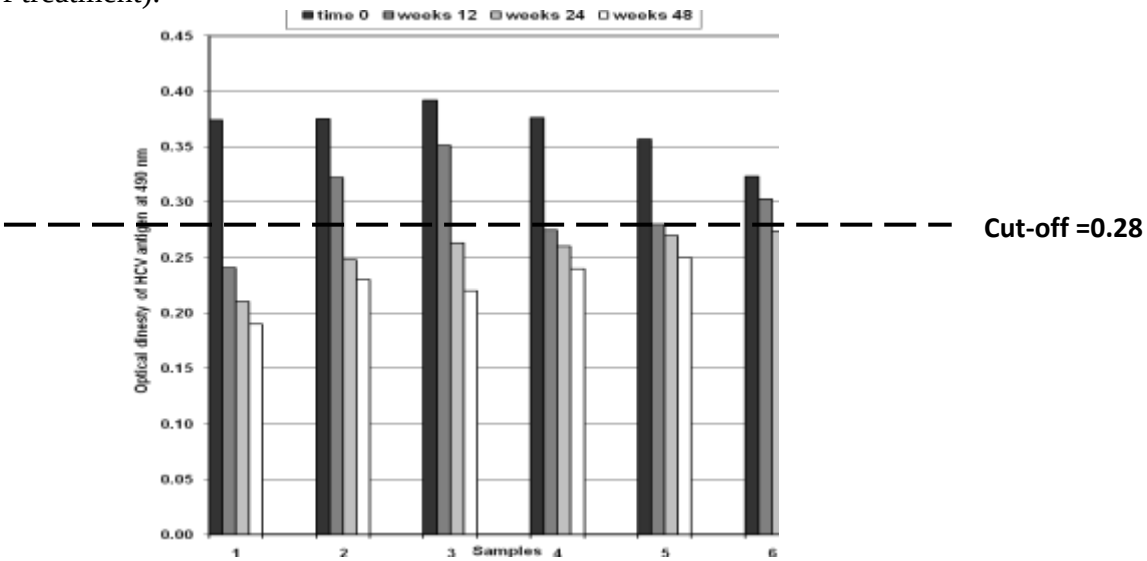

Fig. 7: Responder patients for treatment. Correlation between monitoring the response to HCV therapy (0, 12, 24 and 48 weeks) and viral load presented by HCV antigen (O.D) in 6 responder patients. The detection of HCV antigen showed that (60 %) were responsive for treatment. *Cut-off level of HCV antigen using ELISA: at optical density = 0.28***.**

DISCUSSION:

HCV antigens have been successfully detected using commercial or noncommercial monoclonal and polyclonal **(Nayak et *al.*, 1999)** antibodies directed to core **(Kashiwakuma *et al.*, 1996)** and nonstructural proteins **(Tsutsumi *et al.*, 1994).** The HCV polyprotein is processed by the host and viral proteinases to generate at least 10 functional viral proteins including: core (C), envelope E1, E2, p7, non-structural antigens (NS) 2, NS3, NS4A, NS4B, NS5A, and NS5B **(Grakoui *et al.*, 1993; Bartenschlager and Lohmann 2000).** Many of the HCV NS proteins have been localized to the endoplasmic reticulum (ER) membrane and are essential for productive viral RNA replication **(Kim *et al.*, 1999).** Both HCV NS4A and NS4B proteins play a crucial role in the viral life-cycle and in the development of an immune response to HCV **(Bartenschlager and Lohmann 2000; Masalova *et al.*, 2002 and Gretton *et al.*, 2005)** The diagnosis of acute phase of infection by HCV as well as during reinfections may be achieved through the identification and detection of native antigens and could pave the way for early treatment and consequently effective control of the disease. The antigenic determinants of native non structural protein (NS4) have been identified by using monocolonal antibodies (MAb). However, some MAb and polyclonal antibodies to recombinant proteins and synthetic HCV peptides do not react with native viral antigens **(Masalova *et al.*, 2002)**. Recently, a highly sensitive and specific antibody to a recombinant HCV-NS4 antigen was generated for simple and rapid detection of the target serum antigen using a newly developed dot-ELISA **(Attallah *et al.*, 2003).** In the present study, the circulating non structural protein (NS4) was identified in sera from haemodialysis patients infected with HCV as well as chronic hepatitis C patients at 27 KDa which is similar to the HCV NS4B protein molecular weight **(Konan *et al.*, 2003).** The expression profile of HeLa cells which are stably transfected by HCV non-structural protein 4B was identified by using a specific monocolonal antibody in immunoblot technique at a molecular weight of 27-KDa **(Zheng *et al.*, 2005).** Similarly **Maillard *et al.*, (2001**) had used two monocolonal anti HCV antibodies :the anti-core VT MAb was from Valbiotech (Paris, France; immunogen not communicated) .The bound antigen was detected with the horseradish peroxidase (HRPO)-conjugated MAb ACAP27, with orthophenylenediamine as the substrate. A_{492} was determined with a Titertek Multiscan ELISA plate reader. They also generate MAb 1/1 by immunization of mice with nonenveloped nucleocapsids isolated from plasma of asymptomatic HCV carriers. This antibody was recognized amino acid sequence (aa 45 to 68) of the core protein. Furthermore **Ndiémé *et al.*, (2011)** were used unique monoclonal antibody (mAb) D32.10 which recognizes three highly conserved segments in E1 (aa297-306) and E2 (aa480-494 and aa613-621) envelope glycoproteins. By western blot analysis they found that the mAb D32. 10 recognizes E1E2 envelope complexes expressed in the cell cytoplasm and on the surface of HCV RNA-containing particles released from short-term cultures of in vivo infected hepatocytes. Recently, **El-Shahat *et al.*, (2011)** identified HCV antigen in sera from hepatocellular carcinoma (HCC) patients infected with HCV at 27-KDa molecular weight which contains several epitopes of both linear and conformation-dependent nature. Also **Mosa *et al.*, (2010)** used Western blot analysis showing a single immunoreactive band in cord, CSF, urine and serum of HCV infected patients at 27-KDa. In the present study, an indirect ELISA technique was used for the detection of the circulating nonstructural protein (antigen) in chronic hepatitis C and haemodialysis sera of Egyptian patients. 80 % (60/75) of chronic hepatitis C patients were positive for the target 27-KDa HCV antigen using ELISA. The remaining 20 % (15/75) were negative for HCV antigen using the same technique**.** The detection rate of HCV antigen was very obvious in chronic hepatitis C patients (80%) comparing with those of healthy individuals. Also, the results

revealed that the detection rate of HCV antigen in 75 haemodialysis patients was shown to be 36 % (27/75). Whereas 64 % (48/75) were negative for HCV antigen using ELISA technique. The rate of HCV infection among HD patients shows great variation **(Alavian, 2009).** This variation in HCV infection among different centers may be attributed to the difference in durations that these patients were on maintenance dialysis and on the preventive strategies implemented by different centers against nosocomial transmission of HCV **(Wafaa *et al.*, 2011).** The high level of HCV infection detected in the present study may reflect the high prevalence of HCV infection among HD patients in Egypt. Furthermore, it was found that all healthy individuals used, in this study, as a control were 100 % (25/25) negative for HCV antigen. In the present study we evaluate the usefulness of the detection of HCV antigen using ELISA for therapeutic follow-up (at 0 times, 12, 24 and 48 weeks) in selected 10 positive HCV-RNA patients at 0 times. The detection of HCV-NS4 antigen for these 10 positive HCV-RNA samples at (12 weeks and 24 weeks) showed that 6/10 (60 %) were responsive for treatment and it was found that 4/10 (40 %) did not respond. Currently, it is not obvious whether patients with chronic HCV-G4 respond differently to PEG-IFN-á and ribavirin therapy. Clinical trials conducted in Egypt and the Middle East **(Kamal *et al.*, 2007; Alfaleh *et al.*, 2004 and Kamal *et al.*, 2005)** indicate that the use of PEG-IFN á-2a (180 mcg/week) or PEG-IFN á-2b (1.5 mcg/kg/week) plus ribavirin (1000-1200 mg/day) for 48 weeks results in SVR rates of between 65.8 and 69% **(Kamal *et al.*, 2007).** Further studies are required to investigate the impact of ethnicity, HCV-G4 subtype or the mode of transmission on treatment outcomes in patients with genotype 4.

REFERENCES

Alavian, S.M. (2009): A shield against a monster: Hepatitis C in hemodialysis patients. *World J Gastroenterol.* 14; 15(6):641-646.

Alfaleh FZ, Hadad Q, Khuroo MS, et al (2004): Peginterferon a-2b plus ribavirin compared with interferon a-2b plus ribavirin for initial treatment of chronic hepatitis C in Saudi patients commonly infected with genotype 4. *Liver Int.* 24:568-74.

Attallah AM, Abdel-Aziz MM, El-Sayed AM, Tabll AA (2003a): Detection of serum p53 protein in patients with different gastrointestinal cancers. *Cancer Detect Prev.* 27(2): 127-131.

Attallah AM. Ismail H, Shiha GE, Abou- Dobaraa MI, El-Sherbiny RE, El- Dosoky I (2008): Immunochemical identification and partial characterization of a native hepatitis C viral non-structural 4 antigen in sera of HCV . *Clinica Chimica Acta* 388 (1-2): 115-122.

Bartenschlager R, Lohmann V (2000): Replication of hepatitis C virus. *J Gen Virol.* 81:1631-48.

Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J (1990): Rapid and simple method for purification of nucleic acids. *J Clin Microbio.* 28(3):495-503.

Dennis Revie, Ravi S Braich, David Bayles, Nickolas Chelyapov, Rafat Khan, Cheryl Geer, Richard Reisman, Ann S Kelley, John G Prichardand S Zaki Salahuddin (2005): Transmission of human hepatitis C virus from patients in secondary cells for long term culture. *Virology Journal*. 2:37.

Dragon E (2000): Blood screening by nucleic acid amplification technology: current issues, future challenges. *Mol Diagn.* 5(1):11-22.

El-Shahat A. Toson, Tamer E. Mosa and Mohamed Maher (2011): Identification and detection of a hepatitis C virus antigen in sera of patients with hepatocellular carcinoma. *Nature and Science.* 9(3).

Fabrizi F, Lunghi G, Aucella F, Mangano S, Barbisoni F, Bisegna S, Vigilante D, Limido A Martin P (2005): Novel Assay Using Total Hepatitis C Virus (HCV) Core Antigen quantification for Diagnosis of HCV Infection in Dialysis Patients. *J Clin Microbiol.* 43 (1):414-420.

Fung J, 5*Error! Hyperlink reference not valid.*5Lai CL, Yuen MF (2009): Hepatitis B and C virus-related carcinogenesis. Clin Microbiol Infect. 15(11):964-70.

Grakoui A,Wychowski C, Lin C, Feinstone SM, Rice CM (1993): Expression and identification of hepatitis C virus polyprotein cleavage products. *J Virol.* 67:1385-95.

Gretton SN, Taylor AI, McLauchlan J (2005): Mobility of the hepatitis C virus NS4B protein on the endoplasmic reticulum membrane and membraneassociated foci. *J Gen Virol.* 86:1415-21.

Hinrichsen H, Leimenstoll G, Stegen G, Schrader H, Fölsch UR, Schmidt WE; PHV Study Group (2002): Prevalence and risk factors of hepatitis C virus infection in haemodialysis patients: a multicentre study in 2796 patients. *Gut.* 51(3):429-33.

Jimenez AP, Mohamed MK, Eldin NS, Seif HA, El Aidi S, Sultan Y, Elsaid N, Rekacewicz C, El-Hoseiny M, El-Daly M, Abdel-Hamid M, Fontanet A (2009): Injection Drug Use Is a Risk Factor for HCV Infection in Urban Egypt. *Plos one.* 28;4(9):e 7193.

Kamal S, Kamary S, Shardell M, et al (2007): Pegylated interferon alpha-2b plus ribavirin in patients with genotype 4 chronic hepatitis C: the role of rapid and early virologic response. *Hepatology.* 46: 1732-40.

Kamal SM, El Tawil AA, Nakano T, et al (2005): Peginterferon alpha-2b and ribavirin therapy in chronic hepatitis C genotype 4: impact of treatment duration and viral kinetics on sustained virological response. *Gut.* 54: 858-66.

Kashiwakuma, T.; Hasegawa, A.; Kajita, T.; Takata, A.; Mori, H.; Ohta, Y.; Tanaka, E.; Kiyosawa, K.; Tanaka, T.; Tanaka, S.; Hattori, N.; Kohara, M (1996): Detection of hepatitis C virus specific core protein in serum of patients by a sensitive fluorescence enzyme immunoassay. *J. Immunol. Meth.* 190 (1), 79-89.

Kim JE, Song WK, Chung KM, Back SH, Jang SK (1999): Subcellular localization of hepatitis C viral proteins in mammalian cells. *Arch Virol* .144:329-43.

Konan KV, Giddings TH, Jr., Ikeda M, Li K, Lemon SM, Kirkegaard K (2003): Nonstructural protein precursor NS4A/B from hepatitis C virus alters function and ultrastructure of host secretory apparatus. *J Virol.* 77(14): 7843-7855.

Laemmli UK (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 227(5259): 680-685.

Lee SR, Baggett D, Wright D, Error! Hyperlink reference not valid., ***Walsh I, Busch MP (2005):*** Performance of ORTHO HCV core antigen and trak-C assays for detection of viraemia in pre-seroconversion plasma and whole blood donors. *Vox Sang.* 89(4):201-7.

Masalova OV, Lakina EI, Abdulmedzhidova AG, Atanadze SN, Semiletov YA, Shkurko TV, Burkov AN, Ulanova TI, Pimenov VK, Novikov VV, Khudyakov YE, Fields H, Kushch AA (2002): Characterization of monoclonal antibodies and epitope mapping of the NS4 protein of hepatitis C virus. *Immunol Lett.* 83(3): 187-196.

Mosa TE, Abd Elmaksoud MD Hawas M; Abo-Zeid MM (2010): Assessing possible Different routes of HCV transmission. Nature and Science. 8(12)

Nayak, N.C.; Sathar, S.A (1999): Immunohistochemical detection of hepatitis C virus antigen in paraffin embedded liver biopsies from patients with chronic liver disease. *Acta Histochem.* 101 (4), 409-419.

Ndiémé Ndongo, Subjini Selliah, Pascale Berthillon, Valérie-Ann Raymond, Christian Trépo, Marc Bilodeau, Marie-Anne Petit (2011): Expression of E1E2 on Hepatitis C RNA-Containing Particles Released from Primary Cultured Human Hepatocytes Derived from Infected Cirrhotic Livers. *Intervirology.* 54(1):1-9.

Ray SC, Arthur RR, Carella A, Bukh J, Thomas DL (2000): Genetic epidemiology of hepatitis C virus throughout egypt. *J Infect Dis.* 182(3):698-707.

Ré V, Gallego S, Treviño E, Barbás G, Domínguez C, Elbarcha O, Bepre H, Contigiani M (2005): Evaluation of five screening tests licensed in Argentina for detection of hepatitis C virus antibodies. *Mem Inst Oswaldo Cruz.* 100 (3):303-307.

Sambrook, J., Fritsch, E.F. and Maniatis, T. Gel electrophoresis of DNA. In: Sambrook, J., Fritsch, E.F. and Maniatis, T. (Eds.) (1989): Molecular Cloning: a Laboratory Manual. *New York: Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA*, chapter 6 .

Towbin H, Staehelin T, Gordon J (1979): Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci U S A.* 76(9): 4350-4354.

Tsutsumi, M.; Urashima, S.; Takada, A.; Date, T.; Tanaka, Y (1994): Detection of antigens related to hepatitis C virus RNA encoding the NS5 region in the livers of patients with chronic type C hepatitis. *Hepatol.* 19 (2), 265-272.

Wafaa M. El-Emshaty, Douaa Raafat, Doaa M. Elghannam, Niveen Saudy, Ehab E. Eltoraby, Abd Elhameed A. Metwalli (2011): Diagnostic Performance Of An Immunoassay For Simultaneous Detection Of HCV Core antigen and antibodies among haemodialysis patients. *brazilian Journal of Microbiology*. 42: 303-309.

www. Hawaii. Edu. John A Burns School of medicine University of Hawaii at Manoa. Prevention of hepatitis c infection in Egypt reducing transmission by reducing exposure.

Xie L, Wu XD, Huang DZ, Chen HL, He LX, Wang J, Han DK (2007): Clinical application and analysis of hepatitis C virus NS3 antigen detection by ELISA in human serum. *Chin Med J (Engl).* 120(4):294-9.

Zheng Y, Ye LB, Liu J, Jing W, Timani KA, Yang XJ, Yang F, Wang W, Gao B, Wu ZH (2005): Gene expression profiles of HeLa Cells impacted by hepatitis C virus non-structural protein NS4B. *J Biochem Mol Biol.* 38(2): 151-160.

الكشف علي البروتين غير الهيكلي لفيروس التهاب الكبد الوبائي (سي) في المرضي المصابين بالتهاب الكبد الوبائي المزمن ومرضي الغسيل الكلوي

احمد بركات بركات [١]- حسام الدين احمد غانم[١] – راشد ابراهيم راشد [١]- عبد الفتاح محمد عطا الله[٢]

قسم الميكرو بيولوجى – كلية العلوم – جامعة عين شمس[١]
مركز ابحاث التكنولوجيا الحيوية – دمياط الجديدة[٢]

تعتبر الاصابه بفيروس التهاب الكبد الوبائي (سي) واحده من أهم أسباب الاصابه بأمراض الكبد المزمنه.وتعد الأصابه بالتهاب الكبد الوبائي (سى) مشكله كبيره فى مرضى الغسيل الكلوى وقد تم تحديد عوامل الخطوره فى هؤلاء المرضى الناتج عن عدد مرات نقل الدم أو مدة الغسيل الكلوى. ويتم الكشف الروتينى عن الأجسام المضاده للفيروس الريبوزى فى المراحل المبكره لتأكيد الأصابه ولكنه غير دقيق حيث لا تتكون تلك الأجسام المناعيه فى المراحل المبكره – بينما الكشف عن الحامض النووى الريبوزى للفيروسات فيلزمه تقنيات حديثه مثل تفاعل البلمره المتسلسل (PCR) ويفتقد الي الواقعيه بسبب التكلفه العاليه للاختبار فى الدول الناميه مثل مصر. تهدف الدراسه الحاليه الي التعرف على البروتين غير التركيبى الدائر فى الدم لفيروس التهاب الكبد الوبائى للمرضى المصابين بالفيروس باستخدام اختبار الفصل الكهربى عن طريق الجيل واختبار ويسترن بلوت واختبار الاليزا فى مرضى الغسيل الكلوى والمرضى الغير المصابين بالفيروس كمجموعه حاكمه. وتهدف الدراسه الى تقييم أهمية تعيين البروتين الدائر لفيروس التهاب الكبد الوبائى (سى) بالاليزا فى المتابعه العلاجيه (عند المراحل الزمنيه ٠-١٢-٢٤-٤٨ أسبوع) من خلال تتبع ١٠ أفراد ايجابى فحص تقنية تفاعل البلمره المتسلسل بدايه من المرحله الزمنيه (٠) . تم جمع ٧٥ عينه مصليه من من المرضى المصابين بفيروس التهاب الكبد الوبائى (سى) و ٧٥ عينه مصليه من مرضى الغسيل الكلوى و ٢٥ عينه مصليه من الافراد غير المصابين وهى الشريحه الضابطه. ولقد تم تعريف البروتين الدائر لفيروس التهاب الكبد الوبائى (سى) باستخدام تقنية الفصل الكهربى عن طريق الجيل و طريقة ويسترن بلوت عند ٢٧ كيلو دالتون فى المرضى المصابين بالتهاب الكبد الوبائي (سى) ومرضي الغسيل الكلوى المصابين بالفيروس.وعن طريق استخدام الاليزا تم الكشف علي البروتين غير الهيكلى لفيروس (سى) بنسبة ٨٠% فى المرضى المصابين بالتهاب الكبد الوبائي (سى) وما نسبته ٣٦% فى مرضى الغسيل الكلوى ولم يتم الكشف على البروتين غير الهيكلي فى أى حاله من الشريحه الضابطه. وعند تتبع ١٠ حالات مصابين بالتهاب الكبد الوبائى (سى) من بداية العلاج بالعقاقير المضاده للفيروس (الانترفيرون والريبافارين) وحتى ٤٨ أسبوع من خلال أجراء فحصي تقنية البلمره المتسلسل (PCR) و الكشف على البروتين غير الهيكلى لفيروس (سى) باستخدام الاليزا وجدنا ان ٦ حالات (بنسبة ٦٠%) عند الاسبوع ١٢ و ٢٤ قد استجابوا للعلاج بينما ٤ حالات (اى نسبة ٤٠%) لم يستجيبوا للعلاج .