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Hepatitis C seropositivity and RNA detection among Type2 Diabetic patients in Sulaimani Governorate-Iraqi Kurdistan Region

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Abstract
The current study included 421 cases (221 with type 2 diabetic) and (200 non-diabetics-control group). ELISA and conventional PCR techniques were used for detection of anti-HCV antibodies and HCV-RNA respectively. The percentage rates of HCV seropositivity were (9.5%) among type 2 diabetic patients that were significantly higher ($p < 0.05$) compared to controls. Moreover, HCV-RNA detection was more frequent among diabetic patients (5.88%), although no significant differences found with non-diabetic cases ($p > 0.05$). No significant effects of sex found among positive results ($p > 0.05$). Duration of diabetes, residence, the job type, the patient's education level, history of jaundice, smoking and alcohol consumption showed significant effects ($p < 0.05$) on the percentage rates of HCV infection among type 2 diabetic patients. It was concluded that these parameters are a risk factor for HCV infection among Type 2 Diabetic patients. Moreover, diabetes itself can consider as a risk factor for HCV infection.

Introduction

In the 1970s, a post-transfusion hepatitis causing agent was observed in many countries [1,2]. It turned out to be a virus of the Flaviviridae family, and it referred to as non-A, non-B (NANB) hepatitis, which named as hepatitis C virus (HCV) [3]. After a short time of it's discovery, it was concluded that hepatitis C infection handled more than 90% of cases that named as NANB hepatitis cases. The HCV infection is transmitted mainly through parenteral routes, especially through blood transfusion, resulting in acute or chronic hepatitis.

As more than two third of the newly infected individuals fail to clear the virus during the acute phase and become chronic carriers [4]. The chronic infection, mainly affecting the liver that may cause cirrhosis, ultimately lead to liver failure or hepatocellular carcinoma [5]. The Hepatitis C Virus commonly can spread by blood and blood products, and rarely by sexual transmission [6]. Viremia occurs around seventh days after the first introducing of the virus [7]. The initial infection causes an acute phase of the disease, lasting in average for about 40-70 days, where most of the infected individuals are asymptomatic [8]. In the beginning, the viral infection is detectable by elevated alanine aminotransferase (ALT) levels due to hepatocyte damage. Moreover, the viral nucleic acid can be detectable in the serum by RT-PCR [7, 9, 10]. There is a significant importance to check for HCV nucleic acid in the serum samples of patients suffering from chronic diabetes; A new study revealed that chronic HCV patients who develop diabetes are at increased risk of Liver cancer (hepatocellular carcinoma-HCC) over time [11]. They concluded that patients with new onset diabetes had a significantly higher incidence of HCC as compared to controls. Because diabetes is still an independent predictor for HCC, it is necessary to perform more studies to determine the exact relations between diabetes and HCV infection or HCC. Different studies found that the prevalence rates of HCV infections were higher among diabetic patients when compared with non-diabetic patients. Recently it was discovered by *[12]* that

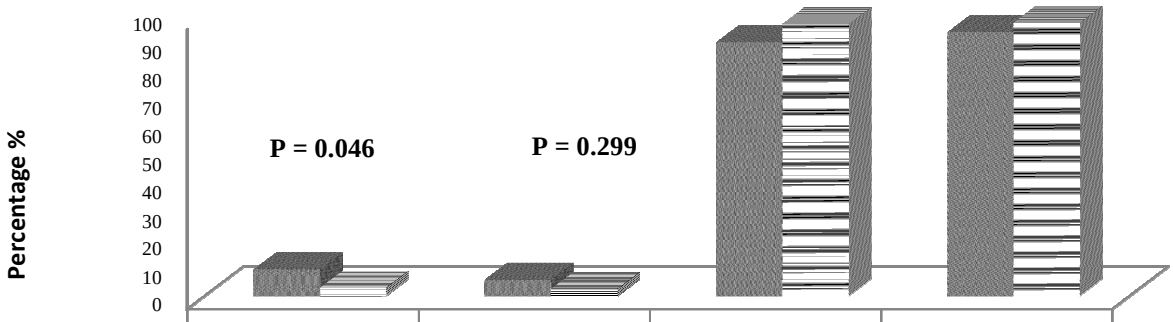
HCV seroprevalence was higher among diabetic patients than among controls. The current study is aimed to detect and determine the seropositivity of anti-HCV antibodies and HCV RNA among type 2 diabetic patients in Sulaimani City, Iraqi Kurdistan region.

Materials and Methods:

In the current study (421) blood samples were collected from both Type 2 diabetic (221) and non-diabetic (200) cases (control group) in Sulaimani City from Dec 5, 2014, to May 15, 2015. A questioner form was used to obtain information from each studied subjects. Serum samples screened for anti-HCV antibodies depending on solid-phase indirect ELISA method (two-step incubation procedure) using Anti-HCV detection Kit (Bioelisa HCV, Biokit, Co. Spain). Screening for HCV RNA was achieved by RNA extraction using conventional PCR technique using (HCV-RNA Extraction, Reverse Transcription and Amplification Kit (QIAGEN). The PCR Detection Kit for HCV is designed to detect RNA extracted from the blood. According to the supplied company instruction RNA extraction, Reverse transcription and RNA amplification protocol was achieved. The gel documentation procedure was done, and the obtained results then tabulated.
Statistical analysis done by using Statigrapic Centurion XVI software (version 16.1.11) and the X^2 test was used.

Results:

Through the current study, it was noticed that the percentage rates of anti-HCV seropositivity were higher among diabetic patients than among nondiabetic cases. Out of 221 diabetic patients, 21 (9.5%) were positive for anti-HCV antibodies whereas 0nly 6 (3%) cases were positive among 200 non-diabetic cases (Fig-1-). Sex showed no significant effects on the obtained results among diabetic and non-diabetic studied cases (p = 0.346 and 0.407) respectively. Significant differences found between diabetic and non-diabetic cases regarding HCV seropositivity (p= 0.047) (Fig.-1-)

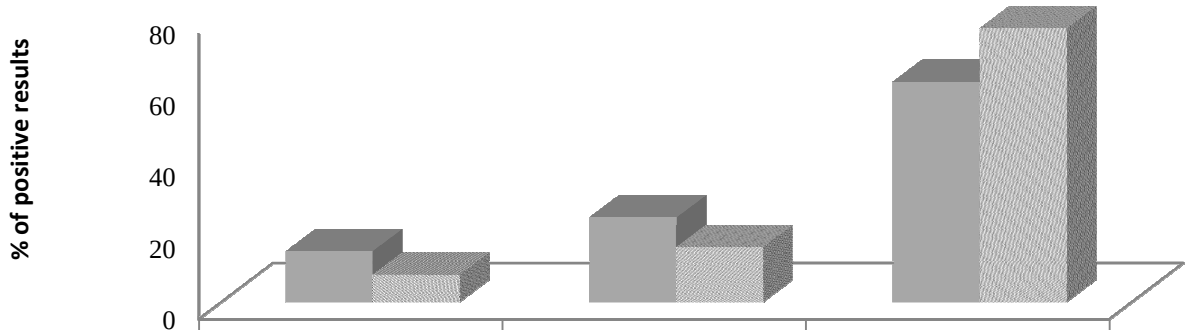


	Positive		Negative	
	Anti-HCV	HCV-RNA	Anti-HCV	HCV-RNA
Diabetic	9.5	5.88	90.5	94.12
Non-diabetic	3	2.5	97	97.5

Figure-1- Perecentages of anti-HCV and HCV-RNA positive results from both Diabetic and non-diabetic cases

Similarly, the percentage rates of HCV RNA positive cases were higher among diabetic (5.88 %) than non-diabetic cases (2.5 %). No significant effects of sexes seen on the obtained results among diabetic patients and non-diabetic cases ((p=0.389, 0.650) respectively (Fig-1-). Moreover, there were no significant differences between obtained results from diabetic and non-diabetic cases regarding nucleic acid detection (p= 0.299) (Fig-1-). The effect of diabetes duration was studied, and the cases divided into three groups. Only 14.3% of cases with diabetes less than five years showed positive results for anti-HCV antibodies while

23.8% of those who suffered from diabetes for 6-10 years were seropositive. The highest rates of seropositive cases (69.1%) observed in patients with type 2 diabetes more than a decade (Fig.-2-). The percentage of HCV RNA positive results were higher (76.9%) among patients with diabetes for more than ten years followed by those who has diabetes for 6-10 years (15.4%).

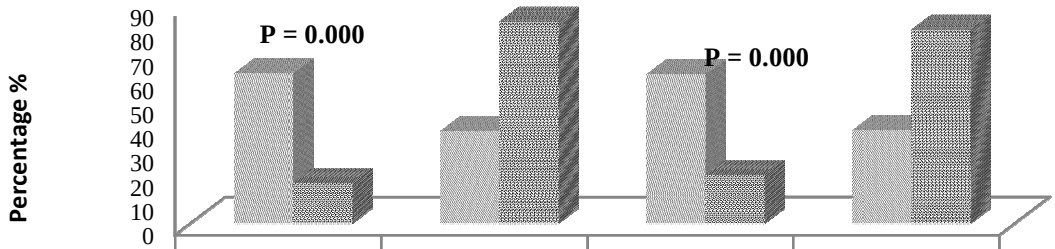


	≥ 5 Years	6-10 Years	> 10 years
Anti-HCV	14.3	23.8	61.9
HCV RNA	7.7	15.4	76.9

Figure-2- Percentage of positive results for both anti-HCV and HCV-RNA accourding to duration of diabetic

The patients with shorter duration of diabetes (less than five years) showed a lower percentage of positive results (7.7%) (Fig.-2-). Diabetes duration showed a significant effect on anti-HCV seropositivity as well as HCV RNA results (p=0.0001, 0.000) respectively. Whereas the diabetic duration showed no significant impact on the positive outcomes regarding anti-HCV and HCV-RNA (p = 0.0695).

Residence showed different effects on the obtained results. Significant effects of residence were reported on the anti-HCV seropositivity among diabetic patients and nondiabetic cases (p= 0.0007, 0.000) respectively. Moreover, the effects were significant on RNA results among both diabetic and non-diabetic patients (p=0.000) for both (Fig. -3-). Residence showed significant effects on obtained results from diabetic and non-diabetic cases at both anti-HCV and nucleic acid detection (p=0.000) for each (Fig.-3-).

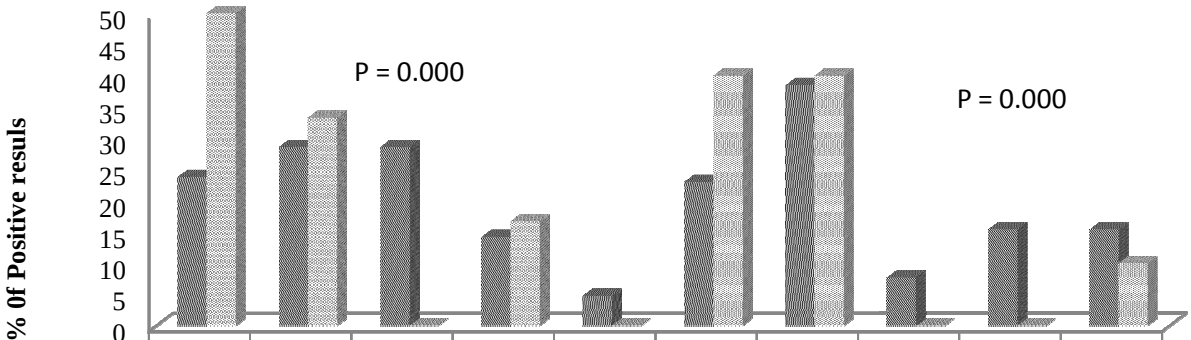


	Urban	Rural	Urban	Rural
	Anti-HCV		HCV-RNA	
Diabetic	61.9	38.1	61.5	38.5
non-diabetic	16.7	83.3	20	80

Figure-3- Effects of residence on anti-HCV and nucleic acid detection among diabetic and non-diabetic cases

The effect of job type of all cases also studied. Observations showed that the job type has significant effects on anti-HCV seropositivity and nucleic acid detection among type 2 diabetic patients and non-diabetic cases

(p= 0.000 for both) where the higher percentage rates of seropositivity were among Employed and students (28.6%, 38.5%) respectively (Fig -4-).

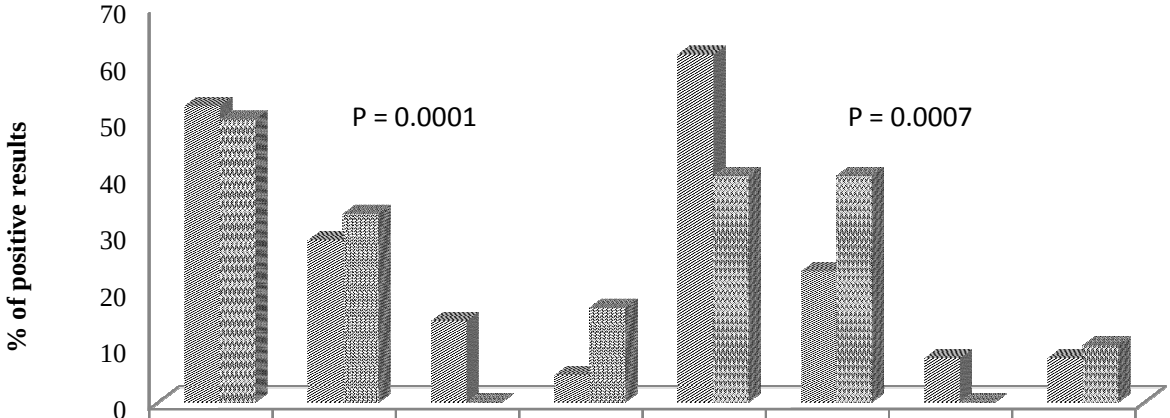


	Worker (service)	Employee	Student	Housewife	Others	Worker (service)	Employee	Student	Housewife	Others
	Anti-HCV					HCV-RNA				
Diabetic	23.8	28.6	28.6	14.2	4.8	23	38.5	7.7	15.4	15.4
Nondiabetic	50	33.3	0	16.7	0	40	40	0	0	10

Figure-4-Effect of job type on anti-HCV seropositivity and RNA detection among diabetic and non-diabetic subjects

Moreover, the effects of education level also were investigated; this study showed that educational attainment has a significant impact on the results among both diabetic and non-diabetic at both anti-HCV seropositivity and HCV-RNA results (P = 0.0001 and 0.0007) respectively (Fig.-5-).

The current study also tried to show the effect of different factors (risk factors) on the anti-HCV seropositivity and HCV-RNA results among diabetic and non-diabetic cases. Smoking, drinking alcohols, history of jaundice and diabetic duration showed significant effects the obtained positive results among diabetic patients and non-diabetic cases (P = 0.000) for both anti-HCV and HCV-RNA results. Moreover drinking alcohol, jaundice history, and diabetic duration showed significant effects on anti-HCV seropositivity between diabetic and non-diabetic cases (P = 0.043, 0.009 and 0.0003) respectively. Whereas smoking and diabetic duration only showed significant effect on HCV-RNA positive results among diabetic and non-diabetic cases (P= 0.000) for both (Table, 1)



	Uneducated	Primary	Secondary	University	Uneducated	Primary	Secondary	University
	Anti-HCV				HCV-RNA			
diabetic	52.4	28.6	14.3	4.7	61.5	23.1	7.7	7.7
non-diabetic	50	33.3	0	16.7	40	40	0	10

Figure-5- Effect of education levels of anti-HCV seropositivity and HCV-RNA among diabetic and non-diabetic cases

Table-1- Effect of risk factors on anti-HCV and HCV-RNA positive results among diabetic and nondiabetic cases

Different Factors		Anti-HCV (%)			HCV-RNA (%)		
		Diabetic	Non-diabetic	P-value	Diabetic	Non-diabetic	P-value
Smoking	Yes	76.2	66.7	0.158	92.3	60	0.000
	No	23.8	33.3		7.7	40	
Alcohol	Yes	71.4	83.3	0.0438	84.6	80	0.352
	No	28.6	16.7		15.4	20	
Marital status	Married	61.9	50	0.087	53.8	60	0.391
	Single	38.1	50		46.2	40	
History of jaundice	Yes	66.7	83.3	0.009	69.2	80	0.074
	No	33.3	16.7		30.8	20	

Discussions:

Diabetic patients are at increased risk to infect with different viruses including hepatitis C virus (HCV). The link between the HCV infections and diabetes was first reported in the nineties of the past century by different researchers [8, 9]. They explained the increased risk of HCV infection among diabetic patients to the frequent parenteral exposures due to the use of finger stick devices, which may expose them to blood borne infections including HCV [10]. From the current study, it was noticed that the percentage rates of anti-HCV seropositivity among diabetic patients were relatively higher in compared to non-diabetic control cases. In a study on diabetic patients in Israel, in 2001, a positive association between HCV infection and diabetes was reported [13], although others found a strong relationship between the raised prevalence of HCV infections and diabetes [14]. Moreover, earlier studies concluded that HCV infection among diabetic patients was 4.39 times higher compared to the control group [15]. Other investigators mentioned similar observations in 2006 and 2007 [16-22], although different researchers reported a lower prevalence of HCV infections among diabetic patients [23, 24]. In recent studies increased prevalence of HCV infections reported [25, 26]. More recently a high prevalence of HCV seropositivity was recorded among diabetic patients in compared with control groups [27]. The current observations are in agreement with most of pre-mentioned earlier reports. The percentage rates of RNA detection were lower than that of anti-HCV antibodies; different factors may contribute and makes RNA undetectable in the patient's serum; including the storage conditions that might destroy RNA since it is very sensitive, technical errors that may happen during PCR technique. Moreover, the major factor may be due to the ability of the immune system to produce neutralizing antibodies that react with the viral RNA, sometimes impair viral replication cycle causing viral RNA undetectable in the circulation in a free form. Current observations agreed with their finding regarding HCV-RNA. They found that the percentage of HCV-RNA positive results were higher among diabetic patients when compared to the obtained results from control groups [28].

Different factors may contribute to the higher percentage rates of anti-HCV seropositivity among patients suffering from diabetic, including daily injection of insulin [20] which makes the diabetic patients parenterally exposed to the risks of infection, as well as using stick devices can expose them to blood borne infections including HCV [10], immunologic problems associated with diabetes that may predispose them to various bacterial and viral infections including HCV.

The current study concluded that sex has no significant effects on the positive results regarding both anti-

HCV antibodies and HCV-RNA, which agreed with earlier results obtained by different researchers [20]. Otherwise, Elhawary *et al.,* in 2011 reported significant effects of sex on HCV infections [25]. In an earlier study, it was reported that the sex (females) is the most common factor for the presence of extrahepatic manifestations of HCV infection [29]. Whereas others found the higher prevalence among males diabetic patients and showed significant effects of sex on HCV seropositivity among diabetic patients [30].

The age showed no significant effects from this study, which was parallel to the observations reported by some investigators [20]. Opposite results found in another study where the most of the seropositive diabetics were in the fifties and sixties of their lives. These observations agreed with a national survey in the US which indicated that HCV infection among diabetic patients more frequently seen in patients older than 40 years [31]. In another previous study in the United States, it was found that the age can consider as a risk factor for clinical and biological extrahepatic manifestations of chronic HCV [29] although the current results agreed with earlier observation [30]. Duration of diabetes showed significant effects on the obtained results from the present study that was incompatible with observations recorded by each of Chen with co-workers [20] and Han with colleagues [26]. Other investigators concluded that duration of a diabetic can consider as an important risk factor for HCV infection, the longer the diabetic duration, the higher the risk. Our observations agreed with results reported in earlier works [30].

The high percentage rates of HCV positive results among rural areas compared to urban may be due to low hygiene among rural inhabitants as well as presence or absence of health centers in the countryside. Moreover, recurrent health checking among cities also can play a role. Current results in agreement with observations recorded in earlier studies that showed significant effects of the locality or residence of the percentage rates of HCV infections among type 2 diabetic patients [25, 30].

Significant effects of the job type observed in the present study that may be due to the frequent exposure to the risks related to HCV including injuries, socioeconomic conditions that can increase the rates of positive results although these factors can affect both diabetic and non-diabetic studied cases equally. Similarly, education level showed significant effects on the obtained results; this may be due to the hygiene levels and the patient educational background about how to protect them from HCV infections, the lower the level of education, the higher the percentage rates of HCV seropositivity. These observations did not agree with findings recorded by [20] who reported no significant effects of education level on the rates of HCV infections among diabetic patients. History of jaundice also was among critically studied factors with significant effects on HCV positive results among diabetic patients which entirely agreed with earlier observations mentioned by Olokoba and colleagues [32]. Moreover, smoking showed significant effects on the current study with noticeable effects on HCV-RNA positive results; this was agreed with previous observations reported by Chuang Shu-Chun *et al.,* 2010 who regarded smoking as a risk factor for HCV infection. Other found that smoking has no significant effects [33].

On the other hand alcohol, consumption may affect the percentage rates of HCV seropositivity among diabetic patients. In a study done in 2006 by Chen and co-workers, it was shown that alcohol drinking can affect noticeably on anti-HCV seropositivity among diabetic patients [20]. Studies showed that alcohol intake with an HCV infection is a health risk which can lead to an increased risk of fibrosis and ultimate cirrhosis [34]. Others concluded that excessive alcohol consumption increase the risk of cirrhosis [35], althoght previous studies reported that alcohol can interfere with the HCV treatments including interferon [36]. Finally this study concluded that HCV infections among diabetic patients may cause different consequences due to the direct effects on the liver. Among the most important outcome is insulin resistance which may be strongly related to the side effects of HCV infection among type 2 diabetic patients. New studies found that HCV infection is independent predictor of type 2 diabetes moreover; statistically significant association of cirrhosis with type 2 diabetes mellitus was reports [37].

References:

[1] Feinstone S. M., Kapikian A. Z., Purcell R. H., Alter H. J., Holland P. V. *"Transfusion-associated hepatitis is not due to viral hepatitis type A or B"*, N Engl J Med, Vol 292, pp 767-770. (1975).

[2] Alter H. J., Purcell R. H., Holland P. V., Popper H. "*Transmissible agent in non-A, non-B hepatitis*" Lancet, Vol. 1, pp 459-463. (1978).

[3] Choo Q. L., Kuo G., Weiner A. J., Overby L. R., Bradley D. W., Houghton M. "*Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome*". Science,Vol. 244, pp 359-362,(1989).

[4] WHO. Weekly epidemiological record: World Health Organization. (1999).

[5] Major M. E., Rehermann B., Feinstone S. M. "*Hepatitis C viruses*". In Fields Virology, Fourth ed, pp. 1127-1161. Edited by D. M. Knipe & P. M. Howley. Philadelphia: Lippincott Williams & Wilkins. (2001).

[6] Clarke A., Kulasegaram R. *"Hepatitis C transmission- where are we now?*" Int J STD AIDS,Vol.17, pp 74-80,(2006)

[7] Shindo M., Di Bisceglie A. M., Biswas R., Mihalik K., Feinstone S. M. "*Hepatitis C virus replication during acute infection in the chimpanzee*", J Infect Dis, Vol. 166, pp 424-427. (1992).

[8] Houghton M. "*Hepatitis C Viruses*". In Fields Virology, 3rd ed, pp. 1035-1058. Edited by D. M. K. B.N. Fields, and P.M. Howley. Philadelphia: Lippincott-Raven Publishers. (1996).

[9] Schlauder G. G., Leverenz G. J., Mattsson L., Weiland O., Mushahwar I. K. "*Detection of hepatitis C viral RNA by the polymerase chain reaction in the serum of patients with post-transfusion non-A, non-B hepatitis*", J Virol Methods, Vol. 37, pp 89-199. (1992).

[10] Marin M. G., Bresciani S., Puoti M., Rodella A., Gussago A., Ravaggi A., Pizzocolo G., Albertini A., "*Mariani E. Clinical Significance of serum hepatitis C virus (HCV) RNA as a marker of HCV infection*", J Clin Microbiol, Vol. 32, pp 3008-3012. (1994).

[11] Knobler H., Schattner A. "*Associaton of Hepatitis C and Diabetes mellitus*", Ann Intern Med. Vol. 132, No.2,pp 141. (2001)

[12] Ryu J. K., Lee S. B., Hong S. J., Lee S. "*Association of chronic Hepatitis C virus infection and diabetes mellitus in Korean patients*". Korean Journal of Intern Medicine, Vol.16, No.1, pp 18-23. (2001).

[13] Simó R., Hernández C., Genescá J., JardÌ R., Mesa J. "*High prevalence of hepatitis C virus infection in diabetic patients*", Diabetes Care, Vol.19, pp 998–1000. (1996).

[14] Sangiorgio L., Attardo T., Gangemi R., Rubino C., Barone M., Lunetta M. "*Increased frequency of HCV and HBV infection in type 2 diabetic patients*", Diabetes Res Clin Pract, Vol. 48, pp 147-51. (2000).

[15] Younas B. B., Khan G. M., Chaudhary M. A. "*Prevalence of diabetes mellitus among patients suffering from chronic liver disease*", Mother and Child,Vol. 38, No.1, pp 37-40. (2000).

[16] Okan V., Araz M., Aktaran S., Karsligil T., Meram I., Bayraktaroglu Z., Demirci F. "*Increased frequency of HCV but not HBV infection in type 2 diabetic patients in Turkey*", Int J Clin Prac, Vol. 56, pp 175-7. (2002).

[17] Bahtiyar G., Shin J. J., Aytaman A., Sowers J. R, McFarlane S. I. "*Association of diabetes and hepatitis c infection: epidemiologic evidence and pathophysiologic insights*", Curr Diab Rep. Vol. 4, No.3, pp 194-198. (2004).

[18] Chen Hua-Fen., Li Chung-Yi., Chen P., See Ting-Ting., Lee Hsin-Yu. "*Seroprevalence of Hepatitis B and C in Type 2 Diabetic Patients*", J Chin Med Assoc, Vol. l69, No. 4, pp 146-152. (2006).

[19] Ali S. S., Ali I. S., Aamir A. H., Jadoon Z., Inayatullah S. "*Frequency of Hepatitis C infection in diabetic patients*", Journal of Ayub Med Coll Abbottabad. Vol. 19, No. 1, pp 46-49. (2007).

[20] Nwokediuko S. C., Oli J. M. *"Hepatitis C virus infection in Nigerians with Diabetes mellitus*". Nigerian Journal of Clinical Practice, Vol. 11, No. 2, pp 94-99. (2008).

[21] Balogun W., Adeleye J., Akinlade K., Otegbayo J. "*Low prevalence of hepatitis-C viral seropositivity among patients with type-2 diabetes mellitus in a tertiary hospital*", J Natl Med Assoc. Vol. 98, No.11, pp 1805-1808. (2006).

[22] Adegoke O. A., Kolawole B. A., Ikem R. T., Adeniran A., Aboderin A. O., Salawu A. "*Seroprevalence of hepatitis C virus infection in Nigerians with Type 2 Diabetes mellitus*", Nigerian Journal of Clinical Practice, Vol.11, No.3, pp 199-201. (2008).

[23] Elhawary E. I., Mahmoud G. F., El-Daly M. A., Mekky F. A., Esmat G. G., Abdel-hamid M. *"Association of HCV with diabetes mellitus: an Egyptian case-control study"*, Virology Journal, Vol.8, pp 367. (2011).

[24] Han Ni., Soe Moe., Aung Htet. *"Hepatitis C Virus Infection in Diabetes Mellitus Patients*", International Journal of Collaborative Research on Internal Medicine & Public Health, Vol. 5, pp 599-606. (2012).

[25] Bhimo T., Laloo D., Prasad L., Ranabir S., Walke P. "*Seroprevalence of hepatitis C infection in type 2 diabetes mellitus*", Indian Journal of Endocrinology and Metabolism, Vol.19, No.2, pp 296-299. (2015).

[26] Kaabia N., Ben Jazia E., Slim I., Fodha I., Hachfi W., Gaha R., Khalifa M., Hadj A. K., Trabelsi H., Abdelaziz A., Bahri F., Letaief A. "*Association of hepatitis C virus infection and diabetes in central Tunisia*", World Journal of Gastroenterology, Vol. 15, No.22, pp 2778-2781. (2009).

[27] Cacoub P., Poynard T., Ghillani P., et al. "*Extrahepatic manifestations of chronic hepatitis C. MULTIVIRC Group. Multidepartment Virus C*", Arthritis Rheum, Vol. 42, No.10, pp 2204-12. (1999).

[28] Jadoon N. A., Shahzad M. A., Yaqoob R., Hussain M., Ali N. "*Seroprevalence of hepatitis C in type 2 diabetes: evidence for a positive association*", Virology Journal, Vol. 7, pp 304. (2010).

[29] Mehta S. H., *et al.* "*Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States*", Ann Intern Med, Vol. 133, No. 8, pp 592-9. (2000).

[30] Olokoba A. B., Badung L. H., Abdulrahman M. B., Salawu F. K., Danburam A., Aderibigbe S., Midala J., Tidi S. K. "*Hepatitis C virus infection in Nigerians with diabetes mellitus*", American Journal of Science and Industrial Research, Vol. 1, No. 2, pp 135-138. (2010).

[31] Shu-Chun, Chuang, Lee Yuan-Chin Amy, Hashibe Mia, Dai Min, Zheng Tongzhang, Boffetta Paolo. "*Interaction between cigarette smoking and hepatitis B and C virus infection on the risk of liver cancer: a meta-analysis*", Cancer epidemiology, biomarkers & prevention, Vol.19, No.5, pp1261-8. (2010).

[32] Huang Y. W, Wang T. C, Yang S. S, Lin S. Y, Fu S. C, Hu J. T, Liu C. J, Kao J. H, Chen D. S. "*Increased risk of hepatocellular carcinoma in chronic hepatitis C patients with new onset diabetes: a nation-wide cohort study*", Aliment Pharmacol Ther., Vol. 42, pp 902–911.(2015).

[33] Laloo D; Walke P; Bhima T; Prasad L; Ranabir S. "*Seroprevalence of hepatitis C infection in type 2 diabetes mellitus*", Indian J Endocrinol Metab. 2015 Mar-Apr, Vol.19, No. 2, pp 296–299. (2015).

[34] Poynard T, Bedossa P, Opolon P. "*Natural history of liver fibrosis progression in patients with chronic hepatitis C*", The lancet, Vol. 349,(9055)m pp 825–832.(1997)

[35] Roudot-Thoraval F, Bastie A, Pawlotsky J. M, Dhumeaux D. "*Epidemiological factors affecting the severity of hepatitis C virus-related liver disease: a French survey of 6,664 patients*", The Study Group for the Prevalence and the Epidemiology of Hepatitis C Virus. Hepatology. 1997 Aug; Vol. 26, No.2, pp 485-90.(1997).

[36] Oshita M1, Hayashi N, Kasahara A, Hagiwara H, Mita E, Naito M, Katayama K, Fusamoto H, Kamada T. "*Increased serum hepatitis C virus RNA levels among alcoholic patients with chronic hepatitis C*", Hepatology. Vol. 20, No.5, pp 1115-1120. (1994).

[37] Memon M. S, Arain Z. I, Naz F, Zaki M., Kumar S., and Burney A. A. "*Prevalence of Type 2 Diabetes Mellitus in Hepatitis C Virus Infected Population: A Southeast Asian Study*", Journal of Diabetes Research; Accepted 15 July 2013 [unpublished].