ABSTRACT: AIM: The present study was done to evaluate the prevalence of hepatitis C virus (HCV) infection in non-insulin dependent diabetic patients (NIDDM) and to investigate influence of HCV seropositivity on several factors such as age of onset of diabetes, complications and mode of treatment. METHODS: The study is prospective, hospital based case - control study done over a period of 3 years. A total of 428 diabetic patients were compared with 1800 voluntary blood donors for the presence of HCV infection. Serological testing for anti HCV was done by using commercial enzyme linked immunosorbent assay (ELISA). Data about various variables were collected from diabetic patients using a structured questionnaire after taking informed consent. RESULTS: Higher prevalence of HCV (7.71%) infection rate observed in NIDDM patients in comparison with blood donors (1.1%). In our study, HCV seropositivity is highest (11.81%) in 40-50 years age group followed by (8.57%) 30-40 years age and least (2.43%) in age > 61 years. Mean age of onset of diabetes mellitus (DM) is 39 years in HCV +ve patients in comparison with 46 years in non-reactors. Proportion of seropositive cases among male and female diabetic cases did not show significant difference. Out of 33 HCV seropositive cases, 63.64% managed with insulin and 48.49% oral hypoglycemic drugs with insulin supplement. Ten out of 33 (30.30%) HCV seropositive cases presented with one or more late diabetic complications compared to 78 out of 395 (19.75%) seronegative cases, with diabetic nephropathy being commonest one. CONCLUSION: Although, HCV infection is more common among adults with type 2 diabetes, it is uncertain whether HCV infection precedes the development of diabetes. Hence, there is a need to perform a prospective analysis to investigate, in persons who acquire type 2 diabetes whether they are more likely to have had antecedent HCV infection. KEYWORDS: HCV, Type 2 diabetes, NIDDM, ELISA.
These days, the number of people with diabetes is increasing due to population growth, aging, urbanization, increasing prevalence of obesity and physical inactivity. Diabetes is rapidly emerging as a global health care problem that threatens to reach pandemic levels by 2030. The number of people with diabetes worldwide is projected to increase from 171 million in 2000 to 366 million by 2030. This increase will be most noticeable in developing countries, where the number of people with diabetes is expected to increase from 84 million to 228 million. According to the WHO, South East Asia and the Western Pacific regions are at the forefront of the current diabetes epidemic, with India and China facing the greatest challenges.

The link between the HCV infection and diabetes was first reported by Allison et al in 1994 and later explored by Simo R et al in 1996. The initial idea that patients with type 2 Diabetes mellitus (T2DM) have more parenteral exposures because of use of finger stick devices and thus are at an increased risk of contracting blood borne infections such as HCV was disproved by a study from France in 1998.

Currently, several studies from different parts of the world have reported that HCV infection may also contribute to the development of diabetes mellitus (DM), and higher prevalence of T2DM has been observed in patients with HCV infection than in those with other forms of chronic hepatitis.

To the best of our knowledge, there are very few documented reports about the prevalence of HCV among diabetic patients in India. Thus, this study is designed to determine and evaluate the prevalence of anti-HCV antibody among diabetic patients as compared to nondiabetic controls.

**MATERIALS AND METHODS:** The study is done over a period of two years in Sree Sidhartha Medical College, Tumkur and Adichunchungiri Institute of Medical Sciences, Bellur. A total of 428 cases (Of all age groups and both sexes) diagnosed as NIDDM/ Type 2 diabetes and on treatment were included in the study. Subjects were excluded if they had type 1 diabetes or emergency cases. The patients who had risk factors for getting HCV infection such as recurrent blood & blood product transfusion, major surgery, intravenous drug abuse, transplantation and dialysis after onset of diabetes were also excluded from the study. Apart from the above exclusion factor, Voluntary blood donors who are known diabetic patients on treatment or with RBS >126 mg/dL and donors with overt liver disease excluded from the study.

The details of the patients such as age, sex, duration of illness, mode of treatment and complications were collected by studying case sheet/ discharge summary and by enquiring patients and physicians using a structured questionnaire.

We have collected 5 ml of venous blood sample in a sterile test tube and left for 30 minutes to facilitate clotting and then the clotted blood was centrifuged to separate the serum from blood. The serum samples were stored in dry clean sterile containers at -20°C prior to use. Serological analysis for the detection of anti HCV antibodies was carried out using rapid immune chromatographic kits. Positive cases were later confirmed by using third generation ELISA kit as per manufacturer’s instruction.

Totally, 1800 voluntary blood donors were taken as control group. Reports of their samples for HCV and HBV infections were collected from blood bank laboratory.
Data analysis was carried out by using SPSS software. Pearson chi-square test was used to determine the difference among various categories with respect to HCV seropositivity. P value of <0.05 was considered statistically significant.

RESULTS: Anti HCV seropositivity is more in study group compared to control group. The difference is statistically highly significant (Table 1). HCV= Chi square value = 64.84/ df =1./ P value <0.001 (Highly Significant).

Anti HCV positivity is highest in 41-50 years age group followed by 31-40 years age group and the least in >61 years. The results are statistically significant for age groups from 31-50 years. Mean age of onset of diabetes in HCV +ve cases is 44 years and in HCV – ve cases is 47 years (Table 2). Chi square value=6.24/df = 3/p value <0.05 (P value is statistically significant).

Anti HCV seropositivity difference between male & female is statistically insignificant (Table 3). P Value > 0.05: Not significant.

Sixty three percent of HCV positive cases needed Insulin/insulin supplement for the management when compared to 27.56% of HCV negative diabetics (Table 4).

Thirty percent of HCV seropositive diabetics presented with late diabetic complications when compared to 19.75% of HCV negative diabetics (Table 5).

| Study Group | Control Group |
|-------------|--------------|
| Total       | 428          |
| Anti HCV positive | 33 (7.71%) |
|             | 20 (1.1%)    |
| Table 1: Comparison of cases and control groups |

| Age group | Total | Hcv +ve | Hcv –ve |
|-----------|-------|---------|---------|
| 30 - 40yrs| 140   | 12(8.57%)| 128(91.43%)|
| 41 - 50yrs| 110   | 13(11.82%)| 97(88.18%)|
| 51 - 60yrs| 96    | 6(6.25%) | 90(93.75) |
| > 61yrs   | 82    | 2(2.44%) | 80(97.56%)|
| Table 2: Age and anti hcv infectivity distribution in type 2 diabetes (study group) |

| Sex      | HCV | HCV +VE | HCV -VE |
|----------|-----|---------|---------|
| Male     | 296 | 23(7.77%)| 273(92.23%)|
| Female   | 132 | 10(7.58%)| 122(92.42%)|
| Table 3: Relationship between sexes with anti hcv seropositivity in type 2 diabetes cases |
**DISCUSSION:** In the present study, higher prevalence of HCV (7.71%) infections observed in NIDDM patients in comparison with blood donors (1.1%). Prevalence of HCV infection compared to control group found to be statistically highly significant. It indicates possible association between NIDDM with HCV infections. However, the study has some limitations, we used HCV cassette antibody test for screening and third generation ELISA to confirm positive cases. We were not able to confirm the results of screening by recombinant immunoblot assay or HCV RNA PCR test due to financial and technical limitations. In addition, we have not collected data on some of the potential confounding factors for developing diabetes like family history, BMI etc. Finally, we do not know the exact pathologic grade and staging of chronic hepatitis as we have not done liver biopsy and ultrasonography on seropositive cases.
The finding of our study exploring higher rate of HCV seropositivity in diabetic patients is in agreement with other studies reported before.\textsuperscript{5,7,8,9,10,11} However, some of the studies also indicated negative association between HCV infection and diabetes mellitus with lower prevalence of HCV in diabetics in comparison with control group.\textsuperscript{12,13} (table 6) The reason for this low prevalence of HCV in diabetes which was seen in these studies might be due to difference in sample size, HCV genotype differences or lower prevalence of HCV in particular community.

In our study, HCV seropositivity in male and females is 7.77% and 7.57% respectively. (p value > 0.05). This shows that there is no significant association between gender and HCV seropositivity in patients with T2DM. This agrees with the work of by Sangiorgio L et al & Ndako JA et al. In study conducted by Sangiorgio L et al, there is no statistically significant difference between hepatitis C positive female (95/846, 11.23%) and male diabetic patients (77/668, 11.53%).\textsuperscript{14} According to Ndako JA et al, The females had 6.3% of positivity, while the males recorded 4.7%. Even though females had a higher prevalence rate, value is not statistically significant.\textsuperscript{15} But in study by Jadoon NA et al, analysis of HCV seropositivity rates with respect to gender revealed that males had higher HCV infection rate of 15.3% as compared to females with 12.4%.\textsuperscript{7}

In the present study, HCV seropositivity is highest (11.81%) in 40-50 years age group followed by (8.57%) 30-40 years age and least (2.43%) in age > 61 years (p value < 0.05). Statistical analysis shows that younger people (30-50 years) have a significantly higher chance of being HCV +ve than older people. Mean age of onset of DM is 44 years in HCV +ve in comparison with 47 years in non-reactors.

In a study done by Wang CS et al, anti-HCV-positive subjects in the age group 35–49 years had three times the prevalence of type 2 diabetes compared with anti-HCV negative subjects followed by age group of 50–64-years (1.6 times).\textsuperscript{16} Mehta SH et al stated that persons 40 years of age or older with HCV infection were more than three times more likely than those without HCV infection to have type 2 diabetes.\textsuperscript{17} According to Ndako JA et al, seroprevalence of HCV among type 2 diabetes cases is highest among age group of 47–57 years (3.3%) followed by those aged > 69 years (3.0%) and 36–46 years age group (2.3%).\textsuperscript{15} On the contrary, Jadoon NA et al, observed that older patients (age > 55 years; 25.3%) were more likely to have HCV infection followed by 46-55 years age group (10.8%) and 36- 45 age group (9.9%). They stated that, the high seropositivity recorded in older group may be because of more parenteral exposures as compared to younger people and thus greater chances of transmission of infection.\textsuperscript{7} Our study denotes that younger diabetics have higher chance of being HCV+ve when compared to older. Even though, this may suggest that HCV +ve cases may develop type 2 diabetes at younger age; it requires prospective study in HCV seropositive patients with multivariate analysis to confirm the same.
In our study, we have noticed that HCV seropositivity in patients with type 2 DM is associated with poor glycemic control. Out of 33 HCV seropositive cases, 21 cases (63.64%) managed with high doses of Insulin (15.15%) and oral hypoglycemic drugs with insulin supplement (48.49%), when compared to seronegative cases (110/395; 27.85%). (12/33) 36.36% of seropositive cases were managed only with oral drugs compared to (285/395) 72.15% in seronegative cases. 10 out of 33 (30.30%) HCV seropositive cases presented with one or more complications compared to 78 out of 395 (19.75%) seronegative cases. Mean age of the patients presenting with complications & mean duration of illness is also slightly lower in HCV +ve when compared to seronegative individuals. This increased frequency of complications in HCV seropositive cases may be due to poor glycemic control in them. Difference between HCV seropositive & seronegative cases in terms of complications is significant with nephropathy (15.5% in HCV +ve & 5.82% in HCV –ve) when compared to others. This could be because of direct involvement of HCV in pathogenesis of glomerulonephritis compounding effect of poor glycemic control.

Even though many studies are there indicating positive association of HCV infection with type 2 diabetes, it is not possible to easily explain the reason for this. The first possibility for this association might be increased exposure to finger prick injury, daily insulin injection and immune compromised state as result of diabetes. The other possibility might be due to the direct and/or indirect effect of HCV infection on glucose metabolism.

According to Decock S et al, The liver is one of the principal organs involved in glucose metabolism together with skeletal muscle and adipose tissue. An obvious and frequently suggested explanation for the connection between HCV infection and type 2 diabetes is that cirrhosis by itself causes insulin resistance. Diabetes developed as a complication of cirrhosis is known as hepatogenous diabetes. According to Picardi A et al, altered glucose tolerance & insulin resistance has been well described in alcoholic chronic liver disease (CLD), non-alcoholic fatty liver disease, chronic hepatitis, hemochromatosis and portal hypertension. Since, in CLD, numerous aetiological, environmental, nutritional and metabolic factors are involved, and the term CLD embraces different stages of liver dysfunction, the pathogenic mechanisms underlying the relationship between IGT or diabetes and CLD remain to be elucidated.

According to these explanations for hepatogenous diabetes, all chronic hepatitis cases caused by infections should have increased risk for development of diabetes. But some of the studies (Mehta SH et al, Decock S et al) have reported significantly greater prevalence of type 2 diabetes in chronic HCV infection, when compared to HBV infection indicating probable direct role of HCV infection in pathogenesis of diabetes.

Mehta SH et al detected diabetes in 24% of HCV infected individuals as compared with 13% of those with HBV infection alone. Decock S et al stated that, the prevalence of type 2 diabetes in HCV cirrhosis is higher than in HBV cirrhosis (23.6% vs 9.4%). Study by Yang SQ et al found that, occurrence of diabetes in patients with chronic hepatitis C was 19.05%, higher than 8.37% in patients with chronic hepatitis B.

At present, plenty of data are available to indicate the direct involvement of HCV in development of type 2 diabetes. Maeno T et al identified hepatic fibrosis and inflammation as key factors to increase insulin resistance in patients with chronic HCV infection. According to Serfaty
L et al, liver fat accumulation and steatosis (due to inhibition of microsomal triglyceride transfer protein by HCV core protein), metabolic conditions such as metabolic syndrome and chronic inflammatory processes in the liver and adipose tissue in chronic HCV infection is responsible for development of diabetes. Elevated levels of pro-inflammatory cytokines such as TNF-α and increased plasma free fatty acids can interfere with insulin signaling through its receptor in persons with chronic HCV, causing insulin resistance. In addition, the HCV core protein can directly inhibit insulin signaling through degradation of insulin receptor substrate (IRS) proteins. HCV core protein can also increase ROS and oxidative stresses, which can further exacerbate insulin resistance (IR).

Aytug S et al found that virtually all HCV-infected patients have early defects in the upstream insulin signaling components like post receptor defect in IRS-1 and insulin signaling defects in hepatic IRS-1 tyrosine phosphorylation and PI3-kinase association/activation that may contribute to insulin resistance.

Knobler H et al stated that even non-diabetic HCV patients have insulin resistance and specific defects in the insulin-signaling pathway by activation of the tumour necrosis factor (TNF)-alpha system. In addition, diabetic HCV patients have significantly higher levels of soluble TNF-alpha receptors, compared to non-diabetic HCV patients and controls.

CONCLUSION: Many epidemiological studies have shown an association between T2DM and HCV infection. The data available from literature provide an understanding of chronic HCV infection, whereby chronic liver diseases like cirrhosis, hepatic steatosis etc., along with insulin resistance and inflammation aggravate each other in a vicious cycle that can ultimately result in HCV-associated DM and its accompanying poor outcomes. Therapies to reduce inflammation, HCV viral load, steatosis and TNF-α activity can therefore be expected to improve metabolic parameters and reduce DM risk in patients with chronic HCV. Further studies are required in this field to develop better strategies to manage diabetes mellitus associated with chronic HCV infection.

The mechanisms by which hepatitis C induces increased insulin resistance and the risk for development of diabetes has not been completely understood. Liver fibrosis progression has long been considered responsible for the appearance of insulin resistance and type 2 DM in patients with chronic liver diseases. The mechanism through which HCV is associated with insulin resistance involves direct viral effects, proinflammatory cytokines and suppressors of cytokine signalling.

In conclusion, there is a significant association between Hepatitis C virus infection and type 2 diabetes in the region according to the findings of the present study. However, it remains to be seen whether diabetes is a risk factor for the HCV infection or vice versa. It is also evident that certain factors including younger age, shorter duration of duration of diabetes with late diabetic complications and poor glycemic control are commonly associated with HCV seopositivity. It requires further prospective studies in patients with chronic HCV infection to look for development of diabetes and follow up to confirm these findings.
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REFERENCES:
1. Alter MJ. Epidemiology of hepatitis C virus infection. World J Gastroenterol 2007; 13: 2436-2441.
2. Rouabhia S, Malek R, Bounecer H, Dekaken A, Amor FB, Sadelaoud M, Benouar A. Prevalence of type 2 diabetes in Algerian patients with hepatitis C virus infection. World J Gastroenterol 2010; 16(27): 3427 – 3431.
3. Wild S, Roglic G, Green A, Sicree R, King H. Global Prevalence of Diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27(5): 1047 – 1053.
4. Allison MED, Wreggitt T, Palmer CR, Alexander GJM. Evidence for a link between hepatitis C virus infection and diabetes mellitus in a cirrhotic population. J Hepatol 1994; 21: 1135 -1139.
5. Simo R, Hernandez C, Genesca J, Jardi R, Mesa J. High prevalence of hepatitis C virus infection in diabetic patients. Diabetes Care 1996; 19: 998 - 1000.
6. Rudoni S, Petit JM, Bour JB, Aho LS, Castaneda A, Vaillant G, Verges B, Brun JM. HCV infection and diabetes mellitus: influence of the use of finger stick devices on nosocomial transmission. Diabetes Metab 1999, 25: 502 - 505.
7. Jadoon NA, Shahzad MA, Yaqoob R, Hussain M, Ali N. Seroprevalence of hepatitis C in type 2 diabetes: evidence for a positive association. Virology Journal 2010; 7: 304.
8. Okan V, Araz M, Aktaran S, Karsligil T, Meram I, Bayraktaroglu Z, Demirici F. Increased frequency of HCV but not HBV infection in type 2 diabetic patients in Turkey. Int J Clin Pract 2002; 56(3): 175 - 177.
9. Yang SQ, Chen HS, Jiang D, Wei L, Ji LN, Wang Y. Relationship between chronic hepatitis C and type II diabetes mellitus. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi 2003; 17(1): 46 - 49.
10. Chen HF, Li CY, Chen P, See TT, Lee HY. Seroprevalence of Hepatitis B and C in Type 2 Diabetic Patients. J Chin Med Assoc 2006; 69(4): 146 – 152.
11. Ali S, Abera S, Mihere A, Abebe T. Association of Hepatitis C Virus Infection with Type II Diabetes in Ethiopia: A Hospital-Based Case-Control Study. Hindawi Publishing Corporation; Interdisciplinary Perspectives on Infectious Diseases. Volume 2012, Article ID 354656, 7 pages. doi:10.1155/2012/354656.
12. Balogun WO, Adeleye JO, Akinlade KS, Kuti M, Otegbayo JA. Low Prevalence of Hepatitis-C Viral Seropositivity among Patients with Type-2 Diabetes Mellitus in a Tertiary Hospital. Journal of the National Medical Association 2006; 98(11): 1805 – 1808.
13. Gulcan A, Gulcan E, Toker A, Bulut I, Akan Y. Evaluation of risk factors and seroprevalence of hepatitis B and C in diabetic patients in Kutahya, Turkey. J Investig Med 2008; 56(6): 858 - 863.
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 2000; 48(2): 147 - 151.

15. Ndako JA, Echeonwu GO, Shidali NN, Bichi IA, Paul GA, Onovoh E, Okeke LA. Occurrence of Hepatitis C Virus infection in type 2 diabetic patients attending Plateau state specialist hospital Jos Nigeria. Virology Journal 2009; 98: 1 – 5.

16. Wang CS, Wang ST, Yao WJ, Chang TT, Chou P. Community-based Study of Hepatitis C Virus Infection and Type 2 Diabetes: An Association Affected by Age and Hepatitis Severity Status. Am J Epidemiol 2003; 158: 1154 – 1160.

17. Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. Ann Intern Med 2000; 133(8): 592 - 599.

18. Decock S, Verslype C, Fevery J. Hepatitis C and insulin resistance: mutual interactions - A review. Acta Clin Belg 2007; 62(2): 111 - 119.

19. Picardi A, D’Avola D, Vespasiani U, Gentilucci, Galati G, Fiori E, Spataro S, Afeltra A. Diabetes in chronic liver disease: from old concepts to new evidence. Diabetes Metab Res Rev 2006; 22: 274 - 283.

20. Maeno T, Okumura A, Ishikawa T, Kato K, Sakakibara F, Sato K, Ayada M, Hotta N, Tagaya T, Fukuzawa Y, Kakumu S. Mechanisms of increased insulin resistance in non-cirrhotic patients with chronic hepatitis C virus infection. J Gastroenterol Hepatol 2003; 18(12): 1358 - 1363.

21. Serfaty L, Capeau J. Hepatitis C, insulin resistance and diabetes: clinical and pathogenic data. Liver International 2009; 29(2): 13 – 25.

22. Aytug S, Reich D, Sapiro LE, Bernstein D, Begum N. Impaired IRS1/PI3-Kinase Signaling in Patients with HCV: A Mechanism for Increased Prevalence of Type 2 Diabetes. Hepatology 2003; 38:1384 - 1392.

23. Knobler H, Schattner A. TNF-{alpha}, chronic hepatitis C and diabetes: A novel triad. QJM 2005; 98 (1): 1 - 6.

24. Romero GM. Insulin resistance and hepatitis C. World J Gastroenterol. 2006; 12: 7075–7080.

25. Douglas MW, George J. Molecular mechanisms of insulin resistance in chronic hepatitis C. World J Gastroenterol. 2009; 15: 4356–4364.

26. Kawaguchi T, Ide T, Taniguchi E, Hirano E, Itou M, Sumie S, Nagao Y, Yanagimoto C, Hanada S, Koga H, et al. Clearance of HCV improves insulin resistance, beta-cell function, and hepatic expression of insulin receptor substrate 1 and 2. Am J Gastroenterol. 2007; 102:570–576.
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