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Seroprevalence and risk factors associated with HBV and HCV infection among subjects with type 2 diabetes from South India

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ABSTRACT

This is a brief summary of the prevalence on Hepatitis C (HCV) and Hepatitis B (HBV) viral infections and associated risk factors in Type 2 diabetes subjects. Prevalence of HBV (9%) was higher compared to HCV (2%) infection in the screened 388 subjects. Results showed that these infections are independent of the liver damage. Risk factors prominently observed among positive HCV and HBV cases were longer duration of diabetes, hospital admission, history of jaundice and history of surgeries which enlightened the importance of hepatitis vaccination once the subject is diagnosed with diabetes.

1. Introduction

Diabetes is an important public health problem which currently affects 425 million adults, and estimated to rise to 629 million by 2045 in the world [1]. Hepatitis C (HCV) and Hepatitis B virus (HBV) infections leads to cirrhosis and hepatocellular carcinoma, and there is a huge burden of these conditions [2]. Prevalence of HCV and HBV infection in India is about 1–1.9% and 2–4.7%. India has over 40 million HBV carriers and accounts for 10–15% of the entire pool of HBV carriers of the world. A study from Tamilnadu showed that overall prevalence of HCV and HBV in general population was 0.2% and 3.6% respectively [3,4]. Glucose metabolism is altered by liver and adequate liver function is essential to maintain glucose homeostasis [5]. Type-2-diabetes (T2DM) is associated with an increased risk of chronic viral hepatitis (CVH). The major risk factors associated with the development of infections are multi-factorial which includes virus-related factors [e.g viral load or genotypes] and host related factors, such as age, gender, alcohol consumption, blood transfusion status, obesity, immune status and co-infections [6]. HCV especially attacks hepatocytes causing acute and chronic type of hepatitis, can be transmitted by blood, sexual contact, contaminated needles or syringes, The likelihood of chronicity after acute HCV infection is as high as 85% with chronic infection being common even in those having normal aminotransferase levels after the acute episode [7] A meta-analysis showed that HCV increases the risk of T2DM by 1.8

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times in excess of that posed by relative degree of liver pathology [8]. On the other hand, HBV can be transmitted via blood or other bodily fluids and people living with T2DM are at an increased risk of contracting HBV infection. The association between positive serology for HBV surface antigen and incident diabetes has been studied in only a few longitudinal studies [9]. It is essential to identify those who are unprotected so that they can be vaccinated [10]. Since the prevalence of diabetes is on the rise and co infection with Hepatitis will make situation worst, screening is very important in high risk subjects for early management. The current study was undertaken to estimate the prevalence of HBV and HCV among T2DM subjects and also to determine the risk factors associated with the condition in bidirectional aspect.

2. Materials and methods

2.1. Study population

This study was a hospital based cross sectional study where a total of 388 (M:F, 215:173) subjects were consecutively enrolled between April 2018 and August 2018 in a tertiary care center for diabetes in Chennai, South India. The subjects were divided into three groups: Group-I T2DM with Abnormal Liver Function test (AbLFT; n = 120), Group-II – T2DM with normal liver function test (NorLFT; n = 138), Group-III – Control with normal liver function and without T2DM [Control; n = 130]. Diagnosis of diabetes was based on the WHO criteria. The study protocol was approved by the institutional ethics committee (Ref No- IEC/N-006/07/2018) and written informed consent was obtained from all the study participants. The study was conducted as per Helsinki's declaration. Sample size was derived based on the preliminary results of pilot study with 30 subjects per group with 95% confidence interval, power of 80%. Patients with type1diabetes, liver cancer, on interferon therapy, having end stage renal disease, gestational diabetes and those who are unwilling to sign the consent form were excluded.

The body mass index (BMI) was calculated. Plasma glucose, serum cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C), and creatinine were measured using fully automated biochemistry autoanalyzers. Glycated hemoglobin A1c (HbA1c) was estimated by high performance liquid chromatography (Bio-Rad, Hercules, CA). All the biochemical investigations were done in an NABL accredited laboratory.

2.2. Quantitative analysis using RT-PCR

HCV RNA was extracted from plasma samples by QIAamp® viral RNA Kit. HCV-RNA was determined quantitatively by real-time PCR assay using Biorad CFX96 T1000 using HCV RT-PCR [Qiagen, USA] quantification kit. Similarly HBV DNA was extracted from plasma sample using QIAamp® mini blood DNA Kit. HBV viral load analysis was done using real-time PCR (HBV RT-PCR (Qiagen) kit).

2.3. Quantitative real-time PCR [qPCR] for HCV analysis

The presence of HCV viral RNA was determined based on the amplification curves. Fluorescence detection channel used were fluorescence Cycling Green FAM and the other is fluorescence ROX channel. Presence of HCV viral DNA was based on positive amplification signals of both the channels.

2.4. Quantitative real-time PCR [qPCR] for HBV analysis

The QIAamp DSP Virus Kit (QIAGEN, cat. no. 60704) was validated for viral DNA purification from human plasma. *artus*® HBV RG PCR kit was used for HBV detection. Fluorescence detection channel used in amplification of HBV were fluorescence Cycling Green FAM and fluorescence Cycling orange HEX channel. Presence of HBV viral DNA was based on both channels showing amplification signals.

2.5. Statistical analysis

Categorical variables were summarized as number (proportion) and continuous variables as mean [standard deviation]. SPSS statistical package (version 20.0; SPSS, Chicago, IL) was used and a P-value less than 0.05 was considered as significant.

3. Results

The mean age of the subjects was 52.7 ± 10.2 years. Differences in the liver function test (AST, ALP, ALT, bilirubin, total protein and serum albumin) was used to classify group I and group II because of the strong evidences on elevated liver enzymes levels during hepatitis infection. The clinical and biochemical characteristics of the study subjects were compared between the groups (AbLFT, NorLFT T2DM and controls). T2DM subjects were older and had high BMI, HbA1c, blood glucose levels, systolic and diastolic blood pressure, creatinine, total cholestrol, triglycerides, levels as compared to control group (Table 1).

Table 2 shows the prevalence of HCV and HBV in the study groups. In total, HBV (9.3%) was more prevalent compared to HCV (2.8%) among subjects. Higher prevalence was seen among men in both HCV (2.6%) and HBV (7.47%) infections as compared to women HCV(0.2%) and HBV(1.8%). The prevalence of HCV in Group I, Group II and Group III were 3.3%, 4.3% and 0.8% respectively. The prevalence of HBV in Group I, Group II and Group III were 5.8%, 20.3% and 0.8% respectively. Higher number of positive cases was observed in groupII as compared to group I. Group II showed the higher prevalence rates of both the hepatitis infections, which in turn proved that elevated liver enzymes are not provoking these infections. Nearly 60% of HCV and 78% HBV infected subjects were having normal liver function tests.

Table 3 shows the clinical and biochemical parameters of HCV and HBV +ve and −ve subjects. Both HBV +ve and HCV +ve subjects were older and had poor glycemic control as compared to −ve subjects. Blood pressure was similar and creatinine levels were elevated in HBV +ve subjects. HbA1c levels were high in HCV +ve cases. The ALP levels were elevated in HCV +ve subjects.

Table 4 shows the distribution of risk factors among HBV and HCV infections. The duration of diabetes, history of hospital admission, history of jaundice, and history of surgeries

Table 1 – Anthropometric and biochemical parameters of the study groups.

Characteristics	Group I T2DM with AbLFT (n = 120)	Group II T2DM with NorLFT (n = 138)	Group III Normal Controls (n = 130)	P value
Age (years)	51 ± 11.9	58 ± 10.9	48 ± 13.2	<0.001
BMI (kg/m^2)	28.0 ± 5.2	26.2 ± 3.6	27.5 ± 5.0	<0.001
HbA1c (%)	8.7 ± 2.0	8.6 ± 1.6	5.6 ± 0.5	<0.001
Urea (mg/dl)	28 ± 15.4	29 ± 12.9	28.3 ± 15.1	>0.05
Serum creatinine (mg/dl)	1.1 ± 0.4	1.19 ± 0.7	1.02 ± 0.3	<0.001
Total cholesterol (mg/dl)	170 ± 47.8	200 ± 66.2	178 ± 36.1	<0.001
LDL chol. (mg/dl)	102 ± 34.4	119 ± 45.3	105.3 ± 24.6	>0.05
HDL chol. (mg/dl)	42.2 ± 31.2	31.2 ± 8.9	48.1 ± 13.1	<0.001
Triglycerides (mg/dl)	155 ± 77.2	170 ± 96.9	122 ± 61.7	<0.001
Fasting glucose (mg/dl)	185 ± 65.28	154 ± 43.2	92 ± 9.7	<0.001
Post prandial glucose (mg/dl)	283 ± 101.6	218 ± 44.21	131 ± 22.4	<0.001
Hb (mg/dl)	13.3 ± 2.0	12.9 ± 2.1	12.6 ± 1.8	>0.05
Blood pressure (mm/Hg)	130 ± 15.3	140 ± 13.1	121 ± 17.5	<0.001
Systolic Diastolic	80 ± 8.2	82 ± 9.1	76 ± 9.9	<0.001

Values are mean ± SD.

Table 2 – Prevalence of HCV and HBV in the study groups

		Total	AbLFT Group I	NorLFT Group II	Control Group III	P-Value	Men	Women
HCV	Positive	11(2.8%)	4 (3.3%)	6 (4.3%)	1(0.8%)	NS	10(2.6%)	1(0.2%)
HBV	Positive	36(9.3%)	7 (5.8%)	28(20.3%)	1(0.8%)	<0.001	29(7.47%)	7(1.8%)

Values are n (%).
NS; non significant.

were remarkably significant in HBV +ve cases whereas no significant difference was seen in the history of smoking, alcohol consumption and family history of DM. Around 80% of those with a previous history of hospital admission had HCV +ve and it was statistically significant [$p < 0.001$]. Prediction of surgical risk is based on the type of surgery and the preclinical status of the patient. In the present study 80% of HCV cases had undergone major surgeries like amputation whereas for HBV infected cases 78% had only day care minor surgery, but what was prominent in both cases was that nearly 90% cases had foot ulcers.

4. Discussion

In this cross-sectional study, the prevalence of HBV and HCV infection were studied and found a higher prevalence of HBV (9.3%) compared to HCV (2.8%). In the present study, older patients were more exposed to the infections. This might be due to more parenteral exposures as compared to young [11]. Seropositivity was more in men compared to women in both HBV and HCV which agree with the work by Caronia et al. [12]. The hypothesis of elevated liver enzymes levels during HBV and HCV infections was totally disproved in this study, where we have found higher rate of seropositivity in diabetes patients with normal liver function test which totally differ from other studies [13,14]. The present study showed only 2.8% of HCV prevalence which was slightly lower than a recent study from north India [15] which reported 5.7% of HCV prevalence among T2DM subjects. The incidence of HCV prevalence was higher in Indian population [16] compared to others [17,18]. Laloo et al. [15] reported that the history of jaundice, blood transfusion, tooth extraction, dialysis, body piercing, tattooing was not significant among HCV positive cases which conflicts with other studies that have hypothesized these risk factors are one of the possible cause of acquiring HCV in T2DM subjects [14,17].

Few studies from USA have reported an increased risk of HBV among persons with diabetes irrespective of HBV risk factors [12,19,20]. Thus, the data Advisory Committee on Immunization Practices [ACIP] has recommended that individuals aged 20–59 years old should get HBV vaccination as soon as they have been diagnosed with diabetes [21]. A study by Uddin et al. [22] found that HBV infection and transmission are geographically more prevalent in Asian regions. Ferreira et al. [23] reported that the risk of incident HBV diagnosis in the diabetes cohort was not different from that in the non diabetes cohort in UK population. However, bayramer et al claim that HBV and HCV associated hospitalization rate was higher in diabetes than in non diabetes cohort [24], which correlate with the present study. The difference in viral infections among T2DM populations may be due to socioeconomical and health care system status of that

Table 3 – Anthropometric and biochemical parameters of hepatitis +ve and -ve subjects.

Characteristics	Hepatitis B virus infection		P-value	Hepatitis C virus infection		P- value
	HBV +ve	HBV -ve		HCV +VE	HCV -VE	
Men: female	29:7	237:115		10:1	256:121	
Age (years)	55.1 ± 7.4	52.5 ± 10.5	<0.001	53.8 ± 10.4	52.4 ± 10.2	<0.001
BMI (kg/m^2)	26.5 ± 4.3	27.2 ± 3.4	NS	25.30 ± 3.3	27.24 ± 3.5	NS
Blood pressure (mm/Hg)	136.5 ± 11.4	130.2 ± 12.7	NS	136.8 ± 14.1	130.6 ± 12.7	NS
Systolic	80.9 ± 6.1	79.4 ± 6.9		81.8 ± 5.6	79.5 ± 6.8	
Dystolic						
Post pandial glucose (mg/dl)	233.2 ± 54.5	206.6 ± 67.6	NS	252.7 ± 40.3	208.3 ± 66.9	NS
HbA1c (%)	9.3 ± 2.1	7.4 ± 1.6	<0.001	8.9 ± 1.4	7.5 ± 1.6	NS
Urea (mg/dl)	32.6 ± 10.6	28.1 ± 9.9	<0.001	32.2 ± 8.6	28.5 ± 10.0	NS
Creatinine (mg/dl)	1.57 ± 0.6	1.06 ± 0.2	<0.001	1.1 ± 0.1	1.1 ± 0.2	NS
Total cholestrol (mg/dl)	152.5 ± 36.1	186.6 ± 38.9	<0.001	148.4 ± 28	184.4 ± 39.7	<0.001
LDL cholesterol (mg/dl)	95.5 ± 24.7	110.4 ± 28.6	<0.001	100.7 ± 22.3	109.2 ± 28.7	NS
HDLcholestrol (mg/dl)	34.0 ± 10.3	40.9 ± 10.3	<0.001	31.8 ± 8.1	40.4 ± 10.4	NS
Triglycerides (mg/dl)	86.7 ± 61.5	155.5 ± 62.0	<0.001	127.2 ± 33.2	116.8 ± 50.5	NS
ALT (IU/L)	25.2 ± 11.5	34.9 ± 14.1	NS	28.9 ± 12.7	34.7 ± 14.1	<0.001
AST (IU/L)	27.6 ± 13.8	43.3 ± 20.8	NS	39.2 ± 16.4	41.9 ± 20.6	NS
Bilrubin (mg/dl)	0.7 ± 0.2	0.7 ± 0.2	NS	0.9 ± 0.3	0.7 ± 0.3	NS
Hemoglobin (%)	12.0 ± 1.5	12.9 ± 1.5	NS	12.1 ± 1.0	12.9 ± 1.6	NS
ALP [IU/L]	241 ± 86.5	199 ± 59.2	NS	261 ± 54.0	201 ± 61.5	<0.001
Total protein (gm/dl)	7.3 ± 0.57	7.9 ± 1.5	NS	6.9 ± 0.4	7.8 ± 1.5	NS
Serum albumin (gm/dl)	3.9 ± 0.5	4.4 ± 0.5	NS	3.6 ± 0.2	4.3 ± 0.6	NS
Serum Globulin (gm/dl)	3.2 ± 0.5	3.1 ± 0.4	NS	2.9 ± 0.5	3.13 ± 0.4	NS

Values are mean ± SD, ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; ALP: Alkaline phosphatase. NS; non significant.

Table 4 – Distribution of various risk factors among HBV and HCV.

Characteristics	HBV [N = 388]		P-value	HCV [N = 388]		P-value
	HBV +VE n = 36	HBV –VE n = 352		HCV +VE n = 11	HBV –VE n = 377	
Duration of diabetes (years)						
<10	10(28%)	119(34%)	<0.001	29(18%)	122(32%)	NS
>10	25(69%)	104(29.5)		8(73%)	126(33%)	
History of Hospital Admission						
Yes	34(94%)	172(49%)	<0.001	10(91%)	196(52%)	NS
History of jaundice						
Yes	30[83%]	165(47%)	<0.001	6(54%)	188(50%)	NS
Family history of diabetes						
Yes	22[61%]	190(54%)	NS	6(54%)	206(55%)	NS
History of surgery						
Yes	28[78%]	28(8%)	<0.001	9(82%)	75(20%)	<0.001
Smoking and Alcohol consumption						
Yes	20(55%)	180 (51%)	NS	6(54%)	206(55%)	NS

Values are number (%).
NS; Non significant.

population as viral infections are mostly blood born, laboratory and selection methods followed will vastly effects the infection rate.

The present study is the first of its kind from South India to evaluate the prevalence and risk factors of HBV and HCV infections. Secondly, we have used quantitative viral load analysis using Real-Time polymerase chain reaction to confirm the presence HCV RNA and HBV DNA. Moreover, we have sub grouped the diabetes patients based on liver enzyme levels to get a more clear results. Control group from same area was used for comparison of findings with the diabetes group. The study also has few limitations. Firstly, the small sample size. The present study did not evaluate the presence of Hepatitis E and A which was also more prevalent in study area and cause of jaundice, and occupational history of the subjects was not noted to rule out any other mode of transmission of viruses. Second, the risk factors were evaluated based on the self-reported medical history which can introduce some degree of misclassification of the outcome status, but this type of misclassification is likely to be

non-differential and therefore, would induce an attenuation of the association.

In conclusion, diabetes was independently associated with an increased risk of hepatitis infections. Persistent mild to moderate elevation of liver enzymes with T2DM cannot be considered as a risk factor for HCV and HBV infection. In addition to these diagnostic considerations, results also conclude that prevalence of HBV is significantly high in the study subjects. The presence of the risk factors such as longer duration of diabetes, history of jaundice, history of frequent hospitalization and history of surgeries make diabetes subjects more susceptible for infections.

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Declaration of Competing Interest

The authors have no conflict of interest to report.

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REFERENCES

[1] IDF Diabetes Atlas. Eighth Edition: International Diabetes Federation; 2017 [10 April 2018]. <https://www.diabete.qc.ca/en/understand-diabetes/. . ./IDFDA-8e-EN-finalR3.pdf>. [accessed 10 Apr 2018].
[2] Mokdad AA, Mokdad AA, Lopez AD, Shahraz S, Lozano R, Mokdad AH, et al. Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. BMC Med 2014;12:145.
[3] Sievert W, Altraif I, Razavi HA, Abdo A, Ahmed EA, Alomair A, et al. A systematic review of Hepatitis C virus epidemiology in Asia, Australia and Egypt. Liver Int. 2011;31(suppl 2):61–80.
[4] Krishnasamy N, Chezhian A, Senthilkumar R, Sathishkumar E. Study of Hepatitis B and C virus infection in urban and rural population of Tamil Nadu, India. Int J Curr Microbiol App Sci 2015;4(6):443–51.
[5] Postic C, Dentin R, Girard J. Role of the liver in the control of carbohydrate and lipid homeostasis. Diabetes Metab 2004;30 (5):398–408.
[6] Garcia-Compean Diego, Jaquez-Quintana Joel Omar, Gonzalez-Gonzalez Jose Alberto, Maldonado-Garza Hector. Liver cirrhosis and diabetes: risk factors, pathophysiology, clinical implications and management. World J Gastroenterol 2009;15(3):280–8.
[7] Nwankiti OO, Ndako JA, Echeonwu GO, Olabode AO, Nwosuh CI, Onovoh EM, et al. Hepatitis C Virus infection in apparently healthy individuals with family history of diabetes in Vom, Plateau State Nigeria. Virol J 2009;6:110.
[8] White DL, Ratziu V, El-Serag HB. Hepatitis C infection and risk of diabetes: a systematic review and meta-analysis. J Hepatol 2008;49(5):831–44.
[9] Huang ZS, Huang TS, Wu TH, Chen MF, Hsu CS, Kao JH. Asymptomatic chronic hepatitis B virus infection does not increase the risk of diabetes mellitus: a ten-year observation. J Gastroenterol Hepatol 2010;25:1420–5.
[10] Asian Liver Center at Stanford University. 2007 Physician's Guide to Hepatitis B: a Silent Killer. 2007 ALC, <http://liver.stanford.edu/files/2007Handbook>.
[11] 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):592–9.
[12] Caronia S, Taylor K, Pagliaro L, Carr C, Palazzo U, Petrik J, et al. Further evidence for an association between non-insulin-dependent diabetes mellitus and chronic hepatitis C virus infection. Hepatology 1999;30(4):1059–63.
[13] Haklar G. liver function tests in chronic viral hepatitis cases. Marmara Med J 2016;29(special issue 1):6–9.
[14] Gray H, Wreghitt T, Stratton IM, Alexander GJ, Turner RC, ORahilly S. High prevalence of hepatitis C infection in Afro-Caribbean patients with type 2 diabetes & abnormal liver function tests. Diabet Med 1995;12:244–9.
[15] Laloo D, Walke P, Bhimo T, Prasad L, Ranabir S. Seroprevalence of hepatitis C infection in type 2 diabetes mellitus. Indian J Endocr Metab 2015;19:296–9.
[16] Lalhriatpuii ST, Sharma AB, Singh KR, Devi KM, Pratima K. Hepatitis C virus seroprevalence among blood alonoes in a tertiary hospital in Manipur. Int J Innov Ros Dev 2014;3:190–2.
[17] 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.
[18] Jadoon NA, Shahzad MA, Yaqoob R, Hussain M, Ali N. Seroprevalence of hepatitis C in type 2 diabetes: evidence for a positive association. Virol J 2010;7:304.
[19] Reilly ML, Schillie SF, Smith E, Poissant T, Vonderwahl CW, Gerard K, et al. Increased risk of acute Hepatitis B among adults with diagnosed diabetes mellitus. J Diabetes Sci Technol 2012;6(4):858–66.
[20] Lanini S, Pisapia R, Capobianchi MR, Ippolito G. Global epidemiology of viral hepatitis and national needs for complete control. J Expert Rev Anti-infective Therapy 2018:625–39.
[21] Advisory Committee on Immunization Practices (ACIP) [Internet]. Available from: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6050a4.html> [Cited 2012 May 14].
[22] Uddin G, Shoeb D, Solaiman S, Marley R, Gore C, Ramsay M, et al. Prevalence of chronic viral hepatitis in people of south Asian ethnicity living in England: the prevalence cannot necessarily be predicted from the prevalence in the country of origin. J Viral Hepat 2010;17:327–35.
[23] Ferreira GL, Marano C, De Moerlooze L, Guignard A, Feng Y, El Hahi Y, et al. Incidence & prevalence of hepatitis B in patients with diabetes Mellitus in the UK: A population-based cohort study using the UK clinical practice research data link. J Viral Hepat 2018;25(5):571–80.
[24] Bayramer HF, Erdem U, Gundogdu TT, cinar Y, Barut Y, Demirtuns R. Prevalence of hepatitis B & hepatis C virus in diabetic patients. Marmara Med J 2001;14(3):160–4.