Original Article

Association of Biochemical Markers between Hepatitis C Virus and Diabetes Mellitus among Bangladeshi Male ∂

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ABSTRACT

Introduction: Diabetes is regarded to be one of the extrahepatic diseases associated with HCV infection. People with diabetes had a higher chance of exposure to HCV. In addition to insulin resistance and glucose intolerance, liver cirrhosis may also result in insulin resistance and glucose intolerance. Objective: The aim of the study is to assess the association of Biochemical Biomarker between Hepatitis C virus and Diabetes Mellitus among Bangladeshi Male. Methods: A total of 197 males suspected of having HCV were included in the study, 111 of whom tested positive through ELSIA and PCR. The analysis indicates that there were 45 (22.8%) HCV-only (without diabetes) participants, 66 (33.6%) HCV-plus-diabetes patients, and 86 (43.7%) diabetes only patients (negative for HCV ELISA). Biochemical tests of all three groups were performed to determine liver, diabetic and lipid profiles. Results: ALT levels were higher in individuals with HCV only and HCV + diabetes than in patients with diabetes alone. ALT was significant among HCV, HCV + diabetes and diabetes only participants (p ≤ 0.005) whereas ALT was highly significant (p ≤ 0.001). AST showed significant to HCV+ diabetes individuals (p ≤ 0.005). Patients with HCV+ diabetes has higher HbA1C, BGF, and BGR levels than patients with diabetes alone. Individual with HCV only showed no statistically significant with HbA1c, BGF and BGR (P> 0.005).For the HCV only group cholesterol differences were extremely significant (P≤ 0.001). For all three patient groups, HDL was found to be non-significant (P > 0.005). Conclusion: Diabetes is significantly associated with hepatitis C virus infection. In this association, serum ALT, triglyceride, and LDL are significant factors. Additional research should shed information on how to enhance the clinical care of diabetic patients with HCV infection.

Key word: Hepatitis C virus, HCV, Diabetes, Biochemical marker

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INTRODUCTION

Up to 1% of the world's population is thought to have HCV infection, which translates to more than 71 million people[1-3]. Asian countries reported seroprevalence rates of 3-4%, whereas central Africa and Egypt reported rates of 10-20%[4]. However, in developing nations, healthcare-associated transmission because of insufficient infection control and prevention continues to be the main pathway for HCV transmission. The majority of incident hepatitis C cases in developed countries are linked to acquisition and transmission among drug users[5,6].HCV primarily affects the liver, but it can also affect tissues outside of the liver, leading to a wide range of extrahepatic symptoms[7]. HCV infections typically start silently and spread to different bodily tissues without showing any overt signs[8]. Patients with HCV infections may experience rheumatologic, dermatologic, diabetic, or renal dysfunction as extra-hepatic symptoms[9].

Diabetes is regarded to be one of the important non communicable diseases, associated with HCV infection. According to Schlimovich et al., people with diabetes had a higher chance of exposure to HCV; the scientists discovered alterations in glucose tolerance in HCV patients[10]. History of HCV infection and severe liver disease, such as cirrhosis and hepatocellular carcinoma, are two major variables in the relationship between diabetes and HCV. In addition to insulin resistance and glucose intolerance, liver cirrhosis may also result in insulin resistance and glucose intolerance[11].Non-alcoholic fatty liver disease, which may lead to cirrhosis and hepatocellular cancer, may be exacerbated by diabetes mellitus. The pace of development into the cirrhotic stage as well as the degree of liver disease brought on by HCV vary greatly. The cirrhotic stage may be influenced by a number of other parameters, including age, gender, alcohol consumption level, obesity, immunological function, and co-infections[12].

A study in Bangladesh conducted by Azam GM., showed the seroprevalence of HCV in type 2 diabetics was 0.48%[13].In Bangladesh, the prevalence of diabetes among men is rising by 10%[14]. Hepatitis C infection's progress is altered by diabetes. Although it is possible for people to acquire insulin resistance on their own, clinical and experimental studies indicate that HCV may have a role in the etiology of the condition[15]. Becausen hepatotoxicity of oral hypoglycemic medications, treating diabetes in cirrhotic patients is much more challenging[16].

OBJECTIVE

The aim of the study is to assess the association of Biochemical Biomarker (ALT,AST) in Hepatitis C virus and Diabetes Mellitus among Bangladeshi Male.

METHOD AND MATERIALS

From January 2020 to December 2022, case-control researchwas carried out in the Outpatients Department of Hepatology and indoor hospitalized patients at IPGMR and BSMMU. The research included 197 male participants who were thought to have HCV.Patients who had previously tested positive for HCV using an HCV quick test kit, who had an abnormal liver function test, or who had any clinical symptoms from BSMMU and BIRDEM had their blood samples obtained. Serum samples for the HCV enzyme linked immunosorbant assay (ELISA), HCV polymerase chain reaction (PCR), liver function tests, diabetic and lipid profiles, as well as for HCV alone, HCV + diabetes, diabetes alone, and control were all examined. The research received institutional review board (IRB) approval and adhered to global standards for investigations involving humans[17]. Patients were fully informed about the trial and gave their informed permission before participating.

Sample collection

An ELISA test for anti-HCV antibodies was conducted using a commercial kit. In accordance with the manufacturer's instructions, RNA from a donor's blood sample was produced, with the phase separation step's centrifugation duration being changed from 15 minutes to 12 minutes. 300 ml of the separated serum were extracted with chloroform and alcohol after being combined with 500 ml of TRIzol reagent. It was reverse transcribed into cDNA using antisense primers after quantification. The five prime untranslated region (5' UTR) was subjected to qualitative PCR using the appropriate primers.

Serum biochemical indicators were analyzed using clinical chemistry kits (ErbaChem 5v3) and an automated biochemistry analyzer (AS-120 Auto Biochemistry Analyzer). Among the biochemical markers examined were the diabetic profile, which included fasting blood glucose (FBG), random blood glucose (RBG), and glycosylated hemoglobin (HbA1C), as well as the lipid profile, which included cholesterol, triglycerides, high densityipoproteins (HDL), and low-density lipoproteins (LDL). Liver profile was assessed, including total bilirubin, albumin level, alanine aminotransferase (ALT), aspartate aminotransferase (AST).

STATISTICAL ANALYSIS

Data was analysed using SPSS 23 version. Quantitative data was provided as mean ± S.D. The Independent Sample t-test was used to compare the means of two groups, and ANOVA was used to compare the means of more than two groups; P ≤ 0.05 was considered significant, P ≤ 0.001 was extremely significant, and P > 0.05 was not significant.

RESULTS

A total of 197 males suspected of having HCV were included in the study, 111 of whom tested positive through ELSIA and PCR. The analysis indicates that

there were 45 (22.8%) HCV-only (without diabetes) participants, 66(33.6%) HCV-plus-diabetes patients, and 86 (43.7%)diabetes only patients (negative for HCV ELISA). 100healthy participants participated as the control group.

Table 1 shows the characteristics of study group according to age and marital status. Prevalence of patients with HCV, HCV+diabetes and diabetes onlyaccountedamong age group between 45-54 (10.2%), 35-44 (10.7%) and 55-64 (18.3%), respectively. 34.5% of the diabetes only patients, 25.9% of HCV + diabetes and 19.3% of HCV were married.

Table 1: Characteristics of study group

Variable	HCV only (45)		HCV + Diabetes (66)		Diabetes (86)	
	n	%	n	%	n	%
Age						
25-34	2	1.0	9	4.6	3	1.5
35-44	16	3.0	21	10.7	12	6.1
45-54	20	10.2	15	7.6	23	11.7
55-64	7	3.6	13	6.6	36	18.3
65 <	-	-	2	1.0	12	6.1
Marital Status						
Married	38	19.3	51	25.9	67	34.5
Unmarried	7	3.6	15	7.6	19	9.6

When liver function tests (LFTs) were evaluated, ALT and AST levels were higher in individuals with HCV only and HCV + diabetes than in patients with diabetes alone. It was found that HCV + diabetic patients had higher ALT titers than HCV alone individuals. Patients in the HCV + diabetes group had ALT levels that were 26.36 0.36 U/L higher than those in the HCV only group. AST, bilirubin and albumin were non-significant among individuals with diabetes only (p>0.005). ALT was significant among HCV, HCV + diabetes and diabetes only participants (p ≤ 0.005) whereas ALT was highly significant (p ≤ 0.001). AST showed significant to HCV+ diabetes individuals (p ≤ 0.005). Bilirubin and Albumin showed no significant with any of the study groups.

Patients with HCV+ diabeteshas higher HbA1C, BGF, and BGR levels than patients with diabetes alone. Individual with HCV only showed no statistically significant with HbA1c, BGF and BGR (P> 0.005).

The HCV+ diabetes group exceeded the other two groups in terms of serum cholesterol and HDL levels. For the HCV only groupcholesterol differences were extremely significant (P≤ 0.001). On the other hand, LDL was marginally elevated in the HCV only group, somewhat higher in the DM only group, and significantly elevated in the HCV +

DM group. However, there was a significant difference in LDL levels between the HCV + DM and DM only groups (P ≤0.005) where with HCV+ diabetes was highly significant (p≤ 0.001). For all three patient groups, HDL was found to be non-significant (P > 0.005).

Table 2: Association between HCV and diabetes on biochemical parameters

Variables	HCV		HCV + Diabetes		Diabetes only	
	Mean ± SD	**P value**	**Mean ± SD**	**P value**	**Mean ± SD**	**P value**
ALT	104 ± 4.75	0.007	115.34 ±1.45	0.004	24 ± 1.86	0.001
AST	107.52 ± 4.51	0.023	115.32± 1.25	0.002	19.25±0.24	0.451
Bilirubin	1.29± 0.07	0.026	1.15 ±.06	0.014	0.62±0.04	0.318
Albumin	4.01 ± 0.13	0.112	3.87 ± 0.13	0.132	4.72±0.45	0.343
HbA1c	4.11 ± 0.7	0.321	8.40±0.13	0.009	8.13±0.64	0.015
BGF	83.27 ± 1.49	0.563	175.82±2.61	0.003	169.12±2.65	0.005
BGR	165.15 ± 1.56	0.264	195.63±3.21	0.004	180.39±4.35	0.002
Cholesterol	231.45 ± 1.48	0.001	252.71±5.23	0.004	229.48±4.32	0.005
TG	235.19±4.37	0.004	207.21 ± 2.45	0.001	215.63±4.21	0.003
HDL	34.59± 0.09	0.423	35.42±7.32	0.521	33.12±0.09	0.113
LDL	153.37±3.12	0.561	141.74±2.93	0.001	146.2 ± 1.12	0.004

Normal values: alanine aminotransferase (ALT) < 40U/L, aspartate aminotransferase (AST) < 40U/L , Bilirubin < 1mg/dl, Albumin: 3.4-4.8 g/dl , Glycosylated hemoglobin (HbA1C): 4 – 6%, Blood glucose fasting (BGF): 70-110 mg/dl, Blood glucose random(BGR): 80-160 mg/dl , cholesterol < 200 mg/dl, triglycerides 80 - 150 mg/dl , high density lipoproteins (HDL) 35 - 55 mg/dl, low density lipoproteins (LDL) 10 – 140 mg/dl ;**Highly significant P ≤ 0.001, *Significant P ≤ 0.005

DISCUSSION

This study revealed that males aged 45 to 65 were more numerous in Bangladesh. This age group is most susceptible to HCV risk factors and extra-hepatic symptoms. Similarly, a study of Pakistani males revealed same results[18]. Mason et al. found that both age and HCV infection were independent predictors of type 2 diabetes[19]. Fraser et al. also suggested that HCV infection and ageing were independent diabetes risk factors. According to reports, old age is one of the most common risk factors for extrahepatic manifestations of HCV[20]. We found that the increased prevalence of HCV mirrors the increased incidence of diabetes. It was previously revealed that HCV patients have a high likelihood of developing diabetes[21].

It was shown in our study that the HCV + diabetic group had relatively higher levels of ALT and AST than the HCV alone group did. Similar to this, several

studies found that diabetic HCV patients had considerably higher ALT and AST values than non-diabetic HCV patients[22]. Compared to diabetes individuals without HCV infection, serum ALT was shown to be elevated in 73.7% of diabetic HCV patients [23]. HCV diabetic individuals have reported having abnormal liver function tests[7]. ALT levels were higher in 32% of HCV-infected diabetes individuals than in 5% of diabetic patients without HCV infection[24]. ALT levels are typically greater than AST levels, but in people with cirrhosis, this may be the opposite [25].

In our study, HbA1C, BGF, and BGR levels were greater in individuals with HCV+ diabetes than in those with diabetes alone. Based on biochemical indicators and biopsy results, it has been established that HCV patients who develop diabetes have more severe liver damage [26]. In accordance with our findings, Bashir M.F et el.[18]and Mason et al[19]and revealed HCV diabetic patients had significantly higher BGF, BGR, and HbA1C levels than non-diabetic HCV patients did. Thus, HCV infection may have a negative impact on the prognosis for diabetes. Bad renal functioning are a result of a poor prognosis[27]. Diabetes and insulin resistance are linked to HCV infection. Patients with chronic liver disease had a 28% abnormal glucose tolerance rate [23].

It has been suggested that lipid profiles and HCV infection may be related. It has been documented that HCV binds to lipoprotein in plasma and that there is a connection between HCV infection and hypobetalipoproteinemia, a condition marked by a low level of lipoproteins in the blood[28-30]. It has been documented that the generation, secretion, and entrance of HCV by human hepatocytes are related to the cholesterol metabolic pathway[31].Clinical studies have demonstrated an association between HCV infection and a particular lipid pattern, including reduced serum TG [32] and cholesterol levels[33].

CONCLUSION

Diabetes is significantly associated with hepatitis C virus infection. In this association, serum ALT, triglyceride, and LDL are significant factors. Additional research should shed information on how to enhance the clinical care of diabetic patients with HCV infection.

REFERENCES

1. Perz, J.F., Armstrong, G.L., Farrington, L.A., Hutin, Y.J. and Bell, B.P., 2006. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *Journal of hepatology*, *45*(4), pp.529-538.
2. Lee, M.H., Yang, H.I., Lu, S.N., Jen, C.L., You, S.L., Wang, L.Y., Wang, C.H., Chen, W.J., Chen, C.J. and Reveal-HCV Study Group, 2012. Chronic hepatitis C virus infection increases mortality from hepatic and extrahepatic diseases: a community-based long-term prospective study. *The Journal of infectious diseases*, *206*(4), pp.469-477.
3. Blach, S., Zeuzem, S., Manns, M., Altraif, I., Duberg, A.S., Muljono, D.H., Waked, I., Alavian, S.M., Lee, M.H., Negro, F. and Abaalkhail, F., 2017. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. *The lancet Gastroenterology &hepatology*, *2*(3), pp.161-176.
4. Abdel-Hamid, M., El-Daly, M., Molnegren, V., El-Kafrawy, S., Abdel-Latif, S., Esmat, G., Strickland, G.T., Loffredo, C., Albert, J. and

Widell, A., 2007. Genetic diversity in hepatitis C virus in Egypt and possible association with hepatocellular carcinoma. *Journal of general virology*, *88*(5), pp.1526-1531.

5. Blach, S., Zeuzem, S., Manns, M., Altraif, I., Duberg, A.S., Muljono, D.H., Waked, I., Alavian, S.M., Lee, M.H., Negro, F. and Abaalkhail, F., 2017. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. *The lancet Gastroenterology &hepatology*, *2*(3), pp.161-176.

6. Lanini, S., Easterbrook, P.J., Zumla, A. and Ippolito, G., 2016. Hepatitis C: global epidemiology and strategies for control. *Clinical Microbiology and Infection*, *22*(10), pp.833-838.

7. Hussain, R.R., Koukab, G., Qayyum, M., Asim, M. and Khanum, A., 2007. Association of diabetes with hepatitis C. virus (HCV) infected male and female patients along with different risk factors. *International Journal of Agriculture and Biology (Pakistan)*.

8. Salomon, J.A., Weinstein, M.C., Hammitt, J.K. and Goldie, S.J., 2002. Empirically calibrated model of hepatitis C virus infection in the United States. *American journal of epidemiology*, *156*(8), pp.761-773.

9. Wang, C.S., Wang, S.T., Yao, W.J., Chang, T.T. and Chou, P., 2003. Community-based study of hepatitis C virus infection and type 2 diabetes: an association affected by age and hepatitis severity status. *American journal of epidemiology*, *158*(12), pp.1154-1160.

10. Shlimovich, P.B., Zus, B.A. and Evdokimov, A.R., 1977. Clinical picture and pathogenesis of Diabetes Mellitus in Chronic Hepatitis and Cirrhosis of the liver. *ProblemyEndokrinologii*, *23*(4), pp.7-14.

11. Garcia-Compean, D., Jaquez-Quintana, J.O., Gonzalez-Gonzalez, J.A. and Maldonado-Garza, H., 2009. Liver cirrhosis and diabetes: risk factors, pathophysiology, clinical implications and management. *World journal of gastroenterology: WJG*, *15*(3), p.280.

12. Noto, H. and Raskin, P., 2006. Hepatitis C infection and diabetes. *Journal of Diabetes and its Complications*, *20*(2), pp.113-120.

13. Azam, G.M., Bhuiyan, M.T., Hoque, N.M., Rahman, A.M. and Khan, A.A., 2016. Seroprevalence of hepatitis B and C Virus infections among type 2 diabetic patients in a tertiary care diabetic centre in Dhaka City. *Int J GastroenterolHepatolTransplNutr*, *1*(iv), pp.17-22.

14. Al Kibria, G.M., 2021. Prevalence and factors associated with diabetes among Bangladeshi adults: an analysis of demographic and health survey 2017–18. *Diabetes Epidemiology and Management*, *2*, p.100012.

15. Negro, F. and Alaei, M., 2009. Hepatitis C virus and type 2 diabetes. *World journal of gastroenterology: WJG*, *15*(13), p.1537.

16. Lee, W.G., Wells, C.I., McCall, J.L., Murphy, R. and Plank, L.D., 2019. Prevalence of diabetes in liver cirrhosis: A systematic review and meta-analysis. *Diabetes/Metabolism Research and Reviews*, *35*(6), p.e3157.

17. Andorno, R., 2007. Global bioethics at UNESCO: in defence of the Universal Declaration on Bioethics and Human Rights. *Journal of Medical Ethics*, *33*(3), pp.150-154.

18. Bashir, M.F., Haider, M.S., Rashid, N. and Riaz, S., 2013. Association of biochemical markers, hepatitis C virus and diabetes mellitus in Pakistani males. *Tropical Journal of Pharmaceutical Research*, *12*(5), pp.845-850.

19. Mason, A., 2001. Viral induction of type 2 diabetes and autoimmune liver disease. *The Journal of Nutrition*, *131*(10), pp.2805S-2808S.

20. Fraser, G.M., Harman, I., Meller, N., Niv, Y. and Porath, A., 1996. Diabetes mellitus is associated with chronic hepatitis C but not chronic hepatitis B infection. *Israel journal of medical sciences*, *32*(7), pp.526-530.

21. Lecube, A., Hernández, C., Genescà, J., Esteban, J.I., Jardí, R. and Simó, R., 2004. High prevalence of glucose abnormalities in patients with hepatitis C virus infection: a multivariate analysis considering the liver injury. *Diabetes care*, *27*(5), pp.1171-1175.

22. Poordad, F., McCone Jr, J., Bacon, B.R., Bruno, S., Manns, M.P., Sulkowski, M.S., Jacobson, I.M., Reddy, K.R., Goodman, Z.D., Boparai, N. and DiNubile, M.J., 2011. Boceprevir for untreated chronic HCV genotype 1 infection. *New England Journal of Medicine*, *364*(13), pp.1195-1206.

23. Bacon, B.R., Gordon, S.C., Lawitz, E., Marcellin, P., Vierling, J.M., Zeuzem, S., Poordad, F., Goodman, Z.D., Sings, H.L., Boparai, N. and Burroughs, M., 2011. Boceprevir for previously treated chronic HCV genotype 1 infection. *New England Journal of Medicine*, *364*(13), pp.1207-1217.

24. Petit, J.M., Bour, J.B., Galland-Jos, C., Minello, A., Verges, B., Guiguet, M., Brun, J.M. and Hillon, P., 2001. Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. *Journal of hepatology*, *35*(2), pp.279-283.

25. Casqueiro, J., Casqueiro, J. and Alves, C., 2012. Infections in patients with diabetes mellitus: A review of pathogenesis. *Indian journal of endocrinology and metabolism*, *16*(Suppl1), p.S27.

26. Jacobson, I.M., McHutchison, J.G., Dusheiko, G., Di Bisceglie, A.M., Reddy, K.R., Bzowej, N.H., Marcellin, P., Muir, A.J., Ferenci, P., Flisiak, R. and George, J., 2011. Telaprevir for previously untreated chronic hepatitis C virus infection. *New England Journal of Medicine*, *364*(25), pp.2405-2416.

27. Thuluvath, P.J. and John, P.R., 2003. Association between hepatitis C, diabetes mellitus, and race: a case-control study. *The American journal of gastroenterology*, *98*(2), pp.438-441.

28. Thomssen, R., Bonk, S., Propfe, C., Heermann, K.H., Köchel, H.G. and Uy, A., 1992. Association of hepatitis C virus in human sera with β-lipoprotein. *Medical microbiology and immunology*, *181*(5), pp.293-300.

29. Monazahian, M., Kippenberger, S., Müller, A., Seitz, H., Böhme, I., Grethe, S. and Thomssen, R., 2000. Binding of human lipoproteins (low, very low, high density lipoproteins) to recombinant envelope proteins of hepatitis C virus. *Medical microbiology and immunology*, *188*(4), pp.177-184.

30. Schonfeld, G., 1995. The hypobetalipoproteinemias. *Annual review of nutrition*, *15*(1), pp.23-34.

31. Ye, J., 2007. Reliance of host cholesterol metabolic pathways for the life cycle of hepatitis C virus. *PLoS pathogens*, *3*(8), p.e108.

32. Fabris, C., Federico, E., Soardo, G., Falleti, E. and Pirisi, M., 1997. Blood lipids of patients with chronic hepatitis: differences related to viral etiology. *Clinicachimicaacta*, *261*(2), pp.159-165.

33. Marzouk, D., Sass, J., Bakr, I., El Hosseiny, M., Abdel-Hamid, M., Rekacewicz, C., Chaturvedi, N., Mohamed, M.K. and Fontanet, A., 2007. Metabolic and cardiovascular risk profiles and hepatitis C virus infection in rural Egypt. *Gut*, *56*(8), pp.1105-1110.