Prevalence of Diabetes Mellitus in Hepatitis C Patients in Wazirabad Tehsil of Gujranwala District of Pakistan

Shazia shamas* [1]   **Mahjabeen Zafar* [1]**   **Shamaila Irum[1]**   **Lubna Khan[2]**
Haleema Sadia[3]   **Sadia Roshan[1]**   **Tanzil Ur Rahman[4]**
Muhammad Yousaf Khan[5]   **Muhammad Ammar[6]**

[1]Department of Zoology, University of Gujrat, Hafiz Hayat Campus, Gujrat, Pakistan
[2]Department of Zoology, University of Buner Khyber Pakhtunkhwa, Pakistan
[3]Department of Biotechnology, Balochistan University of Information Technology, Engineering and management Sciences
[4]Department of Pathology and Pathophysiology, School of medicine, Zhejiang University, Hangzhou, China
[5]Main Pathology Laboratory, Pakistan Institute of Medical Sciences (PIMS)
[6]Genomic Laboratory, Research and Diagnostic, Rawalpindi, Pakistan
*Both authors have equal contribution
Corresponding author: dr.shazia@uog.edu.pk,mahazafar8@gmail.com, shamaila.irum@uog.edu.pk , lubnazoologist@gmail.com, sadia.roshan@uog.edu.pk, endocrinology90@gmail.com, yousafqau@gmail.com, amar.qau@gmail.com sadiahaleema377@gmail.com
*ORCID ID: https://orcid.org/0000-0003-1688-865X *,https://orcid.org/0000-0002-3805-8617, https://orcid.org/0000-0001-6394-8841 , https://orcid.org/0000-0001-9947-6364 ,https://orcid.org/0000-0002-6350-8077 ,https://orcid.org/0000-0001-7506-9393 , https://orcid.org/0000-0002-1305-5655, https://orcid.org/0000-0002-4571-1063 , https://orcid.org/0000-0002-9961-2394

Abstract:

Hepatitis, a condition of liver's inflammation that can be self-limiting or, in certain chances, it may lead to liver cancer, fibrosis or cirrhosis. Hepatitis viruses mainly cause hepatitis in the world. People with hepatitis C have predominant chances to develop diabetes as HCV virus participates in causing type 2 diabetes. HCV virus causes pathogenesis in two ways: it either directly destroys the β cells of pancreas or contributes to the specific autoimmunity of β cells. The present cross sectional study was done in Wazirabad Tahsil of Gujranwala District to analyze the percentage of patients suffering from hepatitis C who had the risk of diabetes mellitus. For this research work, demographic information and data about any other medical history were collected by using a questionnaire. Blood samples were collected from hospital and real time PCR was performed to measure the viral load and blood sugar was measured by using glucometer. Data were then analyzed by using statistically designed software. A total of 29.33% patients, having hepatitis C, were found to be diabetic in Tehsil Wazirabad. 14.70% male and 38.59% female patients having hepatitis C were diabetic. From results shown that the patients of hepatitis C are at higher risk to develop diabetes, therefore; it is supposed that persons having hepatitis C should regularly visit doctors for routine check-up of diabetes and change their life style to reduce the risk of developing diabetes.

Key words: Diabetes mellitus, Hepatitis C, Liver, Pathogenesis.

Introduction:

Liver is a very important body organ. It performs very essential roles such as filtration of toxic materials from the blood, production of blood clotting chemicals and essential enzymes used for digestion. It also imparts significant function in metabolism of different drugs. It also serves as a storage organ for glucose, and vitamins until body requires them. Unfortunately, the liver is also susceptible to many diseases which can harm itself in many ways. These diseases of the liver can be caused by environmental or genetic factors. Although nature has put greater regeneration

capacity in its cells but a severe injury leads to chronic diseases. Any severe damage to liver cells can cause hepatocellular carcinoma, hepatitis fibrosis and hepatitis (1).

Hepatitis is observed to be a major liver's inflammation. The situation could be self-limiting or it may lead to liver cirrhosis, fibrosis and cancer. The main cause of hepatitis is hepatitis viruses but some toxic substances that cause infections including certain drugs, alcohol and various autoimmune diseases. Hepatitis C infections has 15-40% chances to develop other chronic liver ailments such as liver cancer and cirrhosis. Currently, there is no effective vaccine available for hepatitis C therefore; there are many health hazards worldwide (2).

Hepatitis C virus effects about 150-170 million people in the whole world, that makes an estimate of 1.6 to 2.8% of the whole world population (3). Out of 170 million people that have hepatitis C infection, 40% people are from Asian countries (4, 5). It has been estimated that 3-4 million people are infected with hepatitis C virus each year (6). Hepatitis C virus belongs to the family flaviviridae and genus *Hepacivirus* genus. It is a small virus which is enveloped and has 9.6 kb (1kb=10^3 base pairs) positive sense, single stranded RNA genome that shows both lymphotrophism and hepatotrophism (7).

On the other hand, diabetes mellitus is a non-communicable and most prevalent disease. It has been estimated that it affects 347 million individuals worldwide. In 2015, a total of 415 million people were affected by diabetes (8). As well-known, all food is metabolized into glucose which is then used to generate energy. But in diabetes body is unable to produce energy from glucose.

Diabetes mostly develops when patient's body produces insufficient amount of insulin due to improper functioning of pancreas. Basically, diabetes mellitus is of two types, the first one is the Type I in which defective insulin is produced and is most common in children and young adults. Second is type 2 diabetes in which the insulin produced is either insufficient or defective and unable to move glucose into cells.

Although both types of diabetes are incurable but there are many medicines available which help to keep the blood glucose levels under control. Many studies showed that diabetic complications can be avoided by keeping blood glucose levels in normal range.

However, in case of type 2 diabetes choice of treatment depends upon blood glucose levels of the patient. Under this situation the patient is usually recommended to adopt healthy life style by adding exercise in daily life and eating healthy food (9).

It has been known that the hepatitis C patients have more chances to develop diabetes (10). Hepatitis C virus causes diabetes type 2 by inducing insulin resistance (11). Hepatitis C virus impaired the insulin signaling pathway in the hepatocytes by many pathways that include stimulation of the tumor necrosis factor-α (TNF-α), serine phosphorylation of insulin receptors (IRSk) and over expression of suppressor of cytokines (SOC-3) and SOC-7 induction (12).

Although HCV affects liver but it also disrupts the whole body insulin sensitivity without inducing any type of the metabolic syndrome (13). Insulin resistance (IR) and diabetes can develop at any stage of HCV infection. Multiple mechanisms have been accounted for insulin resistance and development of diabetes in patients with chronic hepatitis C. It promotes IR mainly through interfering with insulin signaling pathway in hepatocytes, increasing inflammatory response with production of cytokines such as TNF alpha and IL-6, increasing oxidative stress and reduction in adiponectin levels which may result in liver steatosis (14-16).

Hepatitis C viral infection interferon alpha therapies also trigger the diabetes. It also results in autoimmune diseases due to immunomodulatory effects of IFN-α. Also, injection of IFNα to healthy persons impaired glucose tolerance and insulin sensitivity, stimulated counter regulatory hormone secretion (17).

However, data regarding trend of the prevalence of diabetes among hepatitis C patients in Pakistan is missing. There is no well-organized system available to regulate and monitor general trends in hepatitis C in Punjab province of Pakistan. Therefore, the present research work was done to find the link between HCV and diabetes. For this purpose, a cross sectional study was done in Wazirabad a Tahsil of Gujranwala district to analyze the percentage of patients suffering from hepatitis C having the risk of diabetes mellitus.

Materials and Methods:

This study was done to find the occurrence of diabetes mellitus in hepatitis C patients (both male and female) visiting civil hospital in Wazirabad Tehsil of District Gujranwala in Punjab province of Pakistan from January, 2018 to July, 2018.

A cross sectional study was performed that includes 1296 participants, out of which 273 were positive with HCV having age group from 25 to 60 years.

Blood samples were collected from both male and female patients attending the hospital (Civil Hospital Wazirabad; DHQ) and labs (Chughtai lab Wazirabad; Hassan lab Wazirabad). To collect data from patients a consent form was developed to ensure that our study is totally voluntary based and we followed all the ethical rules. Socio demographic data of all patients were collected by filling questionnaire including age, gender, and history of exposure of associated factors, type of diabetes and treatments of any disease.

In this study, any patient having liver cancer or tumours in pancreas has been excluded. Blood samples were drawn with the help of Senior Health Supervisor. After collecting blood samples, they were centrifuged within one hour. After centrifugation, serum was collected in the vials and stored at -20°C. Serum was then transported to Genomic Lab (Research and Diagnostic, Rawalpindi) for the analysis. Serum samples were run for RNA extraction, cDNA synthesis and real time PCR for the quantification of HCV viral load by using COBAS AmpliPrep/COBAS Taqman (CAP/CTM HCV Version 2, Roche Molecular System, Pleasanton, CA).

Oncall plus blood glucometer was used to measure blood glucose levels (ACON laboratories, San Diego, USA). Age, weight, height, body mass index, LFTs (serum bilirubin, alkaline phosphatase, AST, ALT) and CBCs (HB, TCL, lymphocytes, monocytes, eosinophil) of all patients have been measured.

Weight of all patients was calculated by using digital weighing machine while, waist size and height of all patients was measured by using inches tape, height measuring scale respectively. Body mass index (BMI) was measured by using height and weight of all patients.

Data Analysis:
The data were analysed by using Graphpad Prism 5 and Microsoft excel 2010. Chisquare test was applied to compare the two percentages to find the level of significance. Results were expressed as mean ± SD/ number and percent.

Results:
Prevalence of diabetes in the studied population
The 1296 participants were included in the present study, out of which 246 (18.98%) were found to be diabetic as shown in Table 1. A total of female participants were 792, of whom 184 (23.23%) were diabetic. And total male participants were 504, of whom 62 (12.30%) were diabetic.

Table 1. Prevalence of diabetes mellitus in the population studied

Subjects	Number	Male	Female
Healthy individuals	1296	504	792
Type 2 Diabetic	246	62	184
Non Diabetic	1050	442	608
Percentage of normal subjects having diabetes	18.98%	12.30%	23.23%

Prevalence of diabetes among HCV positive patients
1296 participants were included in the present study, out of which 273 were suffering from Hepatitis C. 29.67% HCV patients were found to be diabetic as shown in Table 2. In our study total female participants were 171(62.64%) suffering from HCV, of whom 66 (38.59%) were diabetic. And total male HCV patients were 102 (37.36%), of whom 15 (14.70%) were diabetic. These results have shown that HCV was more prevalent in females than males.

Table 2. Prevalence of diabetes mellitus among HCV positive male and female patients

Patients	Number	Male	Female
HCV positive	273	102	171
Type 2 Diabetic	81	15	66
Non Diabetic	192	87	105
Percentage of HCV patients having diabetes	29.67%	14.70%	38.69%

Prevalence of diabetes among HCV negative patients
In the present study, 1023 participants were negative for HCV, out of which 165 (16.12%) were diabetic as shown in Table 3. Female participants negative for HCV were 118 (19%) diabetic. Male HCV negative were 47 (11.69%) diabetic. Chi-square analysis shows that HCV positive patients were more diabetic than that of HCV negative participants. The results of Chi-square analysis were found to be statistically significant as ($p< 0.001$) as shown in Fig 1.

Table 3. Prevalence of diabetes mellitus in patients negative for hepatitis C in a comparable age group

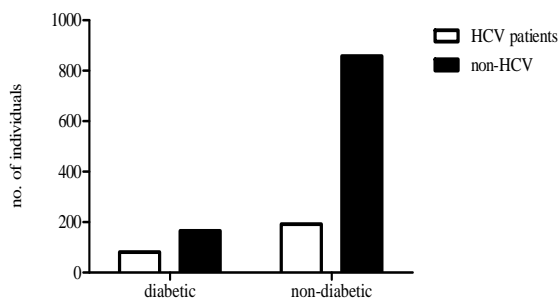| Subjects	Number	Male	Female
Healthy individuals	1023	402	621
Type 2 Diabetic	165	47	118
Non Diabetic	858	355	503
Percentage of normal subjects having diabetes	16.12%	11.69%	19%

Figure 1. Comparison of the 2 percentages using Chisquare test to find the level of significance

Mean viral load of male diabetic patients was 1063157.2±72661 IU/ml, of male non-diabetic was

912462.44±435781 IU/ml, of female diabetic was 1398253±47579 IU/ml and of female non-diabetic was 1266910.5±246145 IU/ml as shown in Fig. 2. Difference between mean viral load of diabetic and non-diabetic HCV positive patients was statistically non-significant. It has also been observed that hepatitis C disease was more prevalent during >30-40 age. Mean Viral loads of total patients were also more in female than in males. Alkaline phosphatases were elevated in both male and female patients of HCV. TCL was also elevated as shown in Table 4.

Table 4. Complete blood picture of all patients suffering from HCV.

| Patients	Normal range	Male		Female	
		Diabetic	Non-diabetic	Diabetic	Non-diabetic
Age		50.4±2.08	38.5±1.72	42.95±1.31	39.78±1.24
Viral load IU/ml		1063157.2±72661	912462.44±435781	1398253±47579	1266910.5±246145
Glucose fasting (mg/dl)	70-110	144±2.09	97.82±2.30	148.33±3.1	104.72±2.93
Weight		82±1	81.31±1.25	79±1.20	73.54±1.1
Serum bilirubin (mg/dL)	0.3-1.2	0.96±0.02	0.84±0.02	0.83±0.01	0.86±0.01
Alkaline phosphatases U/L	35-105	235.4±4.56	228±4.9	220±4.8	236±4.4
AST(U/L)	4-45	46±1.45	37.1±2.4	37±1.1	39.05±1.31
ALT(U/L)	4-42	57.6±2.07	40.75±2.41	40±1.6	44.02±1.9
HB(g/dl)	12-16	12.82±0.26	12.20±0.31	10.33±0.13	11±0.1
TCL/cmm	4000-110000	10680±441	14759±4492	8503±307	18000±3453
Lymphocytes%	25-40	35±1.4	33.86±1.6	34.57±1.37	34.21±1.33
Monocytes%	2-6	4.8±0.39	4.2±0.66	6.19±1.74	3.5±0.78
Eosinophils%	0-4	2.8±0.14	2.82±0.31	2.8±0.19	2.89±0.14
DLC poly		55.6±2.65	61±1.30	63.23±1.41	63.81±1.18
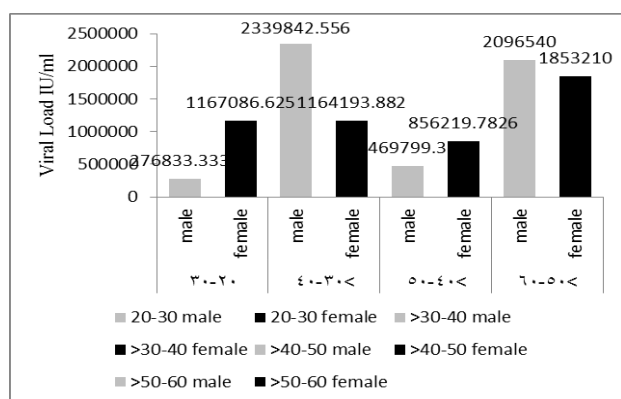
Figure 2. Mean viral load in male and female patients suffering from hepatitis C.

Discussion:

Hepatitis is basically an inflammation of liver and is increasing in Pakistan day by day. Our study found the diabetes prevalence in hepatitis C patients in Wazirabad area of Pakistan. Our study is in accordance with the previous studies which show

strong connection between hepatitis C and diabetes (17). Many studies have reported patients undergone treatment with ribavirin and interferon increased chances of developing Type I diabetes in them (18).

However, the exact mechanism how these antiviral treatments ameliorate the insulin resistance is not fully understood. Nevertheless, it is clear from the previous studies that there is a link between diabetes and hepatitis C. However, the exact mechanism which links HCV and type 2 diabetes is still unclear. Hepatitis C virus may act on pancreas β cell which produce insulin.

Conclusions:

Based on the results it is concluded that persons having heptitis C have more chances to develop type II diabetes than that of normal healthy individuals. Therefore, we suggest that HCV must be treated timely to reduce the chance of developing other diseases likely type II diabetes. However, the

exact mechanism which is involved in the association of these two diseases is still unclear. There may be the chance that hepatitis C virus may affect the liver and pancreas β cell which in turn affect the blood glucose levels. At the end, it can be summarized that Hepatitis C patients are more prone to develop type 2 diabetes therefore; they must be given attention in this regard.

Authors' declaration:
- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Gujrat.

References:
1. Berta E, Egresi A, Bacsárdi A, Gáspár Z, Lengyel G[1], Hagymási K. Hepatitis C virus infection and hepatocarcinogenesis. Orv. Hetil. 2019; 160 (22):846-853.
2. World Health Organization. Hepatitis C, https://www.who.int/news-room/fact sheets/detail/hepatitis-c; 2018 (accessed 18 July 2018).
3. Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. J. Hepatol. 2014; 61(1):45–57.
4. Westbrook RH, Dusheiko G. Natural history of hepatitis C. J. Hepatol. 2014; 61(1Suppl):58–68.
5. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology 2015; 61(1):77–87.
6. Lange CM, Jacobson IM, Rice CM, Zeuzem S. Emerging therapies for the treatment of hepatitis C. EMBO Mol. Med. 2014; 6(1): 4–15.
7. Simmonds P, Becher P, Bukh J, Gould EA, Meyers G, Monath T, et al. Rico-Hesse R, Smith DB,

8. Stapleton JT. ICTV Virus Taxonomy Profile: Flaviviridae. J Gen Virol. 2017; 98(1):2-3.
9. Cho NH. Q&A: Five questions on the 2015 IDF diabetes atlas. Diabetes Res Clin Pract 2016; 115:157–9.
10. Diabetes Research & Wellness Foundation (DRWF). 2016. www.diabeteswellness.net.
11. Vanni E, Bugianesi E, Saracco G. Treatment of type 2 diabetes mellitus by viral eradication in chronic hepatitis C: myth or reality?. Digestive and Liver Disease. 2016 Feb 1;48(2):105-11.
12. Negro F. Facts and fictions of HCV and comorbidities: steatosis, diabetes mellitus, and cardiovascular diseases. J. Hepatol. 2014; 61(1suppl):69–78.
13. Alipourfard I, Datukishvili N, Mikeladze D. TNF alpha Downregulation modifies insulin receptor Substrate 1 (IRS-1) in Metabolic Signaling of Diabetic Insulin-Resistant Hepatocytes. Mediators. Inflamm. 2019: 3560819.
14. Kawaguchi Y, Mizuta T. Interaction between hepatitis C virus and metabolic factors. World J Gastroenterol. 2014; 20(11):2888-2901.
15. Lemoine M, Chevaliez S, Bastard JP, Fartoux L, Chazouillères O, Capeau J et al. Association betweenIL28Bpolymorphism, TNF and biomarkers of insulin resistance inchronic hepatitis C-related insulin resistance. J. Viral. Hepat. 2015; 22(11):890-6.
16. Peta V, Torti C, Milic N, Focà A, Abenavoli L. Adiponectin serum level in chronic hepatitis Cinfection and therapeutic profile. World. J. Hepatol. 2015; 7(1):44-52.
17. Bernsmeier C, Calabrese D, Heim MH, Duong HT. Hepatitis C virus dysregulates glucose homeostasis by a dual mechanism involving induction of PGC1 and dephosphorylation of FoxO1. J. Viral. Hepat. 2014; 21(1):9-18.
18. Hammerstad SS, Grock SF, Lee HJ, Hasham A, Sundaram N, Tomer Y. Diabetes and Hepatitis C: A Two-Way Association. Front. Endocrinol. (Lausanne). 2015; 6:134.
19. Laurito MP, Silva GF, Cheinquer H, Sharma R, Verna E, Parise ER. Does insulin resistance impair the virological response to peginterferon/ribavirin in chronic hepatitis c genotype 3 patients? Arq. Gastroenterol. 2018; 55 (2):179-183.

انتشار داء السكري في مرضى التهاب الكبد الوبائي في وازيراباد تهسيل في مقاطعة جوجرانوالا في باكستان

شازيا شماس[1]   محجبين ظفر[1]   شميلة إيروم[1]   لبنى خان[2]   حليمة سعدية[3]

ساديا روشان[1]   تنزيل اور الرحمن[4]   محمد يوسف[5]   محمد عمار[6]

[1]قسم علم الحيوان، جامعة جوجرات، حرم حافظ حياة، جوجرات، باكستان
[2]قسم علم الحيوان، جامعة بونر خيبر باختونخوا، باكستان
[3]سم التكنولوجيا الحيوية، جامعة بلوشستان لتكنولوجيا المعلومات، الهندسة والعلوم الإدارية
[4]قسم علم الأمراض والفيزيولوجيا المرضية، كلية الطب، جامعة تشجيانغ، هانغتشو، الصي
[5](PIMS) مختبر علم الأمراض الرئيسي، المعهد الباكستاني للعلوم الطبية
[6]Genomic Laboratory، Research and Diagnostic، Rawalpindi،Pakistan

الخلاصة:

التهاب الكبد ، وهو حالة من التهاب الكبد التي يمكن أن تحد من نفسها أو ، في بعض الفرص ، قد تؤدي إلى سرطان الكبد أو التليف الكبد. فيروسات التهاب الكبد تسبب بشكل رئيسي التهاب الكبد في العالم. HCVلديهم فرص سائدة للإصابة بمرض السكري حيث ان فايروس Cالأشخاص المصابون بالتهاب الكبد الوبائي. يشارك في التسبب في مرض السكري من النوع الثاني فيروس التهاب الكبد الوبائيHCV إلى التسبب في بطريقتين: إما أنه يدمر خلايا البنكرياس β مباشرة أو يساهم في المناعة الذاتية المحددة للخلايا. وقد أجريت هذه الدراسة مستعرضة الحالية في وازيراباد تهسيل من منطقة جوجرانوالا لتحليل النسبة المئوية للمرضى الذين يعانون من التهاب الكبد C الذين لديهم خطر الإصابة بداء السكري. بالنسبة لهذا العمل البحثي ، تم جمع المعلومات والبيانات الديموغرافية حول أي تاريخ طبي آخر باستخدام استبيان. تم جمع عينات الدم من المستشفى وتم إجراء PCR في الوقت الحقيقي لقياس الحمل الفيروسي وتم قياس نسبة السكر في الدم باستخدام مقياس السكر. تم تحليل البيانات باستخدام برنامج مصمم إحصائيا. تم العثور على ما مجموعه 29.33 ٪ من المرضى ، الذين يعانون من التهاب الكبد C ، لمرضى السكري في وازيراباد تهسيل. وكان 14.70 ٪ من الذكور و 38.59 ٪ من الاناث المرضى الذين يعانون من التهاب الكبد C يعانون من مرض السكري. تشير النتائج إلى أن مرضى التهاب الكبد الوبائي سي يكونون أكثر عرضة للإصابة بمرض السكري ؛ نفترض أن الأشخاص المصابين بالتهاب الكبد الوبائي سي يجب عليهم زيارة الأطباء بانتظام لفحص مرض السكري بشكل روتيني وتغيير نمط حياتهم لتقليل خطر الإصابة بمرض السكري.

الكلمات المفتاحية: داء السكري، التهاب الكبد الوبائي، الكبد، المرضية.