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Original Article

Frequency of Type 2 Diabetes Mellitus among Patients with Hepatitis C Virus Infection

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ABSTRACT

Background: Hepatitis C virus (HCV) infection and type 2 diabetes mellitus (T2DM) have established themselves as significant global health concerns, with a notable co-prevalence that suggests a potential pathophysiological connection. The increasing incidence of T2DM, particularly in developing countries, alongside the significant burden of HCV, poses challenges for healthcare systems and necessitates deeper understanding of their interrelation.

Objective: This study aimed to ascertain the frequency of T2DM among patients with HCV infection and to investigate the potential association between HCV reactivity and the incidence of T2DM.

Methods: A cross-sectional analysis was conducted at a molecular pathology laboratory in Lahore, Pakistan. Patients aged 18-60 years without cirrhosis or pre-existing T2DM were consecutively enrolled over a six-month period. Exclusion criteria included liver cancer, interferon therapy, end-stage renal disease, other viral hepatitides, and pregnancy. Data were collected via questionnaires after informed consent, with serum and hemoglobin A1c (HbA1c) levels measured. HCV reactivity was confirmed via real-time polymerase chain reaction (RT-PCR), and the frequency of T2DM was assessed by HbA1c criteria. Statistical analysis involved univariate analysis using SPSS version 25.0.

Results: Among the 300 HCV-reactive patients, the frequency of T2DM was found to be 49.33% (148 patients). The mean age of participants was 42.71 ± 6.808 years, with 53.0% males and 47.0% females. Urban and rural participants were nearly equally represented. A significant association was noted between HCV reactivity and T2DM (p=0.001), while no significant relationship was found with gender, residence, marital status, or family history of T2DM.

Conclusion: The study highlights a substantial frequency of T2DM in HCV-reactive patients and a significant association between HCV infection and the development of T2DM. Regular HbA1c monitoring in HCV-reactive patients is recommended for the early identification and management of T2DM.

Keywords: Hepatitis C Virus, Type 2 Diabetes Mellitus, HCV-reactivity, Hemoglobin A1c, Cross-sectional Study, Polymerase Chain Reaction, Insulin Resistance, Epidemiology, Public Health.

INTRODUCTION

Pakistan is at the forefront of countries grappling with a significant burden of hepatitis C virus (HCV) infection and type 2 diabetes mellitus (T2DM), both of which present considerable public health challenges (1). The prevalence rates of HCV and T2DM in Pakistan are among the highest in the world, a situation highlighted by data from the World Health Organization and the International Diabetes Federation, which place Pakistan at the pinnacle of global prevalence for these conditions (2, 3). This dire health scenario can be attributed to a constellation of factors including unsafe medical practices, a healthcare infrastructure that fails to meet the needs of its population, limited access to essential screening and treatment options, a genetic predisposition among the population,

the rapid urbanization leading to significant lifestyle changes, and a pervasive lack of awareness regarding disease transmission mechanisms (4).

Emerging evidence underscores a concerning association between chronic HCV infection and an increased risk of developing T2DM (5-7). This association is thought to arise from the capacity of HCV to induce insulin resistance, a hallmark of T2DM, through both direct and indirect mechanisms. Specifically, proteins encoded by HCV have been demonstrated to disrupt insulin signaling pathways, thereby hindering glucose uptake and utilization by cells. Moreover, the chronic inflammatory state induced by persistent HCV infection, both within the liver and systemically, may further exacerbate insulin resistance, setting the stage for the development of T2DM. The progression of HCV infection towards liver fibrosis, cirrhosis, and ultimately liver failure underscores the virus's capacity to impair liver function, a critical component of glucose metabolism, thus contributing to the emergence of T2DM (8). Additionally, HCV's impact on adipose tissue function, characterized by alterations in adipokine secretion and insulin sensitivity, further predisposes individuals to T2DM. The possibility of shared genetic predispositions further complicates the relationship between HCV infection and T2DM, suggesting a complex interplay of factors that necessitate further investigation (6).

Given these findings, it is imperative that individuals with chronic HCV infection be regularly screened for T2DM, and conversely, those diagnosed with T2DM should be evaluated for HCV infection. Adopting a proactive and integrated approach to the management of both HCV infection and T2DM—encompassing lifestyle modifications, strict adherence to treatment regimens, and consistent medical oversight—can significantly enhance the prognosis and quality of life for affected individuals. Effective management of these conditions is crucial not only for improving individual health outcomes but also for mitigating the risk of further complications. This study aims to elucidate the frequency of T2DM among patients infected with HCV, providing valuable insights into the interrelation of these diseases, and informing future healthcare strategies.

MATERIAL AND METHODS

This cross-sectional study was conducted at the Molecular Pathology Laboratory of Farooq Hospital Westwood, Lahore, and received ethical approval from the Ethical Review Committee of the College of Allied Health Sciences, Akhter Saeed Medical & Dental College, Lahore, ensuring adherence to the ethical guidelines of the Declaration of Helsinki for medical research involving human subjects. The study targeted HCV reactive patients without pre-existing liver cirrhosis and/or T2DM, aged between 18 and 60 years, encompassing both genders, who visited Farooq Hospital Westwood. The recruitment of participants was executed consecutively over a six-month period from August 2023 to January 2024. Exclusion criteria included patients with liver cancer, liver cirrhosis, those undergoing interferon therapy, individuals with end-stage renal disease, coexisting viral infections such as hepatitis B surface antigen positivity, and pregnant females.

Data collection was facilitated through a structured questionnaire, specifically designed for this study, to gather relevant patient information following the acquisition of written informed consent, which was a prerequisite for participation in the study. Venous blood samples of approximately 5ml were collected from all study participants using clotted vacutainers. These samples were subsequently centrifuged at three thousand revolutions per minute for ten minutes to separate the serum, which was then stored at -20°C until DNA extraction. In parallel, about 3ml of venous blood was drawn into ethylenediaminetetraacetic acid (EDTA) vacutainers for the determination of Hemoglobin A1c (HbA1c) levels, adhering to the American Diabetes Association's recommendation of using HbA1c levels ≥6.5% as a diagnostic criterion for T2DM, alongside or as an alternative to fasting plasma glucose criteria (10).

The viral nucleic acid extraction from the serum samples employed a Qiagen nucleic acid extraction kit, following a manual protocol that included the addition of Qiagen proteinase, sample, lysis buffer, absolute ethanol, wash buffers, and elution buffer in specified quantities. The elution of viral nucleic acids was carried out with 60µl of elution buffer. The integrity of these extracted nucleic acids was promptly assessed through a one-step real-time polymerase chain reaction (RT-PCR) using a ZEESAN kit, following the kit's protocol for the preparation of the master mix and subsequent processing in a Rotar Gene-Q 5plex thermocycler. The RT-PCR protocol included initial denaturation and a series of annealing and extension cycles, with the cycler threshold (CT) values set to distinguish between positive (CT value 12 to 19) and negative (CT value greater than 30) HCV cases.

HbA1c levels were measured using the AUDICOM AC6601 system, which operates on the principle of ion-exchange liquid chromatography. The statistical analysis of the data was conducted using the Statistical Package for the Social Sciences version 25.0 (SPSS 25.0). This analysis included the summarization of quantitative variables as means and standard deviations and qualitative variables as frequencies and percentages. A univariate analysis was employed to identify predictive variables associated with the occurrence of T2DM in patients reactive to HCV, ensuring a comprehensive evaluation of the relationship between HCV infection and the development of T2DM.

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RESULTS

The results, in following table, the characteristics of HCV reactive patients are laid out along with the frequency percentages and the results of the univariate analysis. The average age of the participants is around 43 years. There's an almost equal split among the participants' age ranges, with a slight majority in the 40-60 year age bracket. The gender distribution is fairly even with a slight male predominance. Urban and rural residence is evenly split. Most of the participants are married. Approximately half of the patients have Type 2 Diabetes Mellitus, which is the only characteristic that showed a statistically significant association with HCV reactivity, indicated by a p-value of 0.001. There is no significant association between family history of T2DM and HCV reactivity.

Table 1: Demographic and Clinical Characteristics of HCV-Reactive Patients

Characteristics	HCV Reactive Patients Frequency (%)	Univariant Analysis p-value
Mean age (Years)	42.71 ± 6.808	N/A
Age Range 18-40 (Years)	136 (45.33%)	0.980
Age Range 40-60 (Years)	164 (54.66%)	N/A
Gender Male	159 (53.0%)	0.515
Gender Female	141 (47.0%)	N/A
Residence Urban	156 (52.00%)	0.932
Residence Rural	144 (48.00%)	N/A
Marital Status Single	56 (18.66%)	0.908
Marital Status Married	244 (81.33%)	N/A
Type 2 Diabetes Mellitus No	152 (50.66%)	0.001*
Type 2 Diabetes Mellitus Yes	148 (49.33%)	N/A
Family History of T2DM No	191 (63.66%)	0.132
Family History of T2DM Yes	109 (36.33%)	N/A

*A p-value of <0.005 is considered statistically significant.

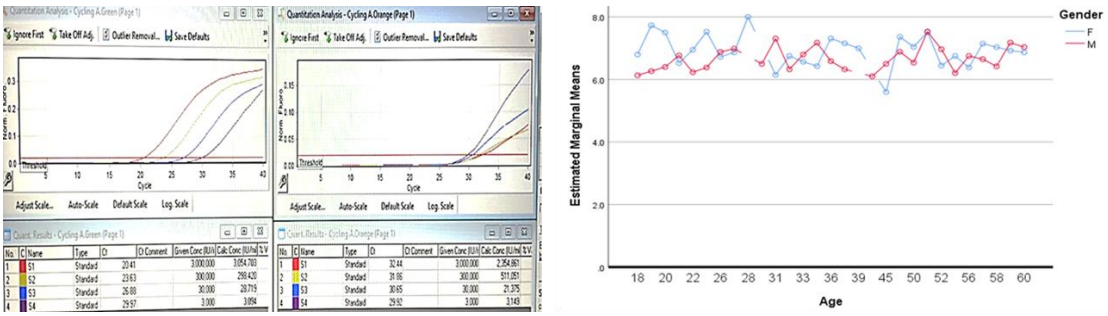

Figure 1 RT-PCR (Real-Time Polymerase Chain Reaction)

The image showing of RT-PCR (Real-Time Polymerase Chain Reaction) quantitation analysis software showing two separate amplification plots, labeled 'Cycling A.Green' and 'Cycling A.Orange'. In both plots, four different samples (S1, S2, S3, S4) are shown, each represented by a distinct curve that amplifies over cycles of PCR, reflecting the quantity of nucleic acid present. For 'Cycling A.Green', the samples cross the threshold line (indicative of detectable product levels) at varying cycle points, with Ct (cycle threshold) values recorded for S1 at 20.41, S2 at 23.63, S3 at 26.08, and S4 at 28.97. The given concentrations for these samples are consistent at 3,000 IU/mL, with calculated concentrations close to this value. In the 'Cycling A.Orange' plot, the crossing points and Ct values differ from 'Cycling A.Green', with S1 at 32.44, S2 at 31.86, S3 at 31.05, and S4 at 28.92 cycles. Here, the given concentration is also 3,000 IU/mL for S1, S3, and S4 but is 300,000 IU/mL for S2, resulting in calculated concentrations that are markedly higher, particularly for S2 at 511,051 IU/mL, indicative of a high amount of viral nucleic acids. The age graph representing estimated marginal means of a certain metric plotted across different ages ranging from 18 to 60 years, separated by gender (F for females and M for males). The graph exhibits fluctuations in the values across age groups for both genders. For instance, at the age of 22, males have a peak value which is above 7, while females show a peak around the age of 31, which is just below 7. On the other hand, at the age of 39, the value for females dips to just above 4, which is one of the lower points for this gender on the graph. Generally, female values fluctuate between just below 5 and 7, while male values vary more widely, approximately between 4 and slightly above 7. This suggests a potential trend or pattern that could be related to the prevalence or severity of a health condition, or possibly a response to treatment, with respect to age and gender.

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DISCUSSION

The discussion of the relationship between hepatitis C virus (HCV) infection and type 2 diabetes mellitus (T2DM) is grounded in the context of their rising prevalence worldwide. The seminal work by Allison et al. in 1994 laid the foundation for this exploration by illustrating an elevated prevalence of T2DM in HCV-reactive cirrhotic patients awaiting transplantation compared to those with cirrhosis due to other liver diseases (13). This correlation was further substantiated by the work of Mehta et al., which indicated that HCV-reactive patients bore a threefold increase in the risk of developing T2DM relative to their non-reactive counterparts (14). These studies collectively affirm the enduring association posited between HCV reactivity and T2DM, though not without contention, as some research has offered contrasting findings (15).

In the current study, the patient demographic comprised a slight majority of males over females, with a substantial proportion being married and a near-equal distribution between urban and rural residence. Crucially, the study discerned no significant correlation between T2DM and gender, residence, marital status, or family history of T2DM (p-values: 0.515, 0.932, 0.908, 0.132 respectively). This stands in contrast to the findings of Siddiqui M et al., which echoed a higher predisposition to T2DM among HCV-reactive patients (16), as well as the work of Elhawary et al., which paralleled these results (17). Despite the nonsignificance of some variables in the current study, the widespread prevalence of both conditions in the general population commands further scrutiny (18), indicating the necessity for more extensive, larger-scale research.

In examining age demographics, this study identifies that a larger segment of HCV-reactive patients fell within the 40-60 years age range, underscoring the potential influence of advanced age on the incidence of HCV-associated T2DM, a finding corroborated by other research in the field (19, 20). The frequency of T2DM in HCV-reactive patients was observed at 49.33% (148 cases), signaling a considerable co-occurrence of T2DM within this patient population and establishing a significant association between HCV reactivity and T2DM (p=0.001). This is in line with Kalar et al., who reported an even higher frequency of T2DM in HCV-reactive patients (21), and Villar LM et al., who observed a heightened prevalence of HCV in T2DM patients, albeit with some variation potentially attributed to the strict inclusion criteria of these studies (22).

The mechanistic linkage between T2DM and HCV may be attributed to factors such as insulin resistance, increased hepatic tumor necrosis factor-alpha, augmented hepatic glucose production, and defects in insulin secretion, with the HCV core-encoding region instrumental in inducing insulin resistance (23). Additionally, pre-existing risk factors for diabetes, such as family history and age, also play a pivotal role in those with HCV infection (24, 25). While the findings are significant, the study is not without limitations. It is based on a relatively small patient cohort, is single-centered, and excludes non-HCV reactive patients, potentially limiting the generalizability of the results.

CONCLUSION

In conclusion, the study observed a high frequency of T2DM among HCV-reactive patients and confirmed a statistically significant association between HCV reactivity and T2DM. It underscores the importance of regular monitoring of HbA1c levels in HCV-reactive patients for the early diagnosis of T2DM and advocates for screening for HCV infection among the diabetic population as a reciprocal measure. Future research should aim to build on these findings with larger and more diverse patient cohorts to provide a more comprehensive understanding of the association between HCV and T2DM.

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