Hepatitis C virus infection in a population with high incidence of type 2 diabetes: Impact on diabetes complications

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Received 2 March 2011; received in revised form 25 May 2011; accepted 26 May 2011

KEYWORDS
Hepatitis C; Type 2 diabetes; Diabetes complications; Viral load; HbA1c; Risk factors

Abstract A growing number of reports suggest a connection between hepatitis C virus (HCV) infection and type 2 diabetes (T2D). However, the association of HCV infection with diabetes-related complications has not yet been clarified. The aim of this study was to determine the prevalence of HCV infection in T2D-patients in Kuwait which has a high incidence of type 2 diabetes, and to investigate the association between HCV viremia and diabetes-related complications. A total of 438 patients with T2D (325 Kuwaitis and 113 Egyptians), and 440 control subjects, were enrolled for this study. HCV infection was assessed by testing for serum HCV-specific antibodies, and by detection of HCV RNA. HCV viral load and hemoglobin A1c (HbA1c) levels were assessed in patients with and without diabetes complications. Thirty one (7%) out of 438 T2D-patients had evidence of HCV infection compared to 4 (1%) out of 440 control adults (p < 0.0001). The prevalence of HCV infection in Kuwaiti and Egyptian T2D-patients was 3% and 18%, respectively. Most of the HCV sequences detected in T2D patients and control subjects were of genotype 4. The HbA1c levels in T2D-patients with HCV viremia were significantly higher than those in HCV-negative patients. HCV viremia, female sex, age, family history of diabetes...
Introduction

Diabetes mellitus is a major public health problem in Kuwait and is one of the fastest growing diseases around the world. It is estimated that 15—21% of the Kuwaiti adult population have type 2 diabetes (T2D) [1,2]. The incidence of diabetes in Kuwait is very high compared with neighbouring Arab countries, and it may be the result of the rapid economic and social development which took place after the oil boom in Kuwait. Family history of diabetes, obesity, diets high in calories and lack of exercise have all been identified as risk factors for developing T2D in Kuwait population [1,2], suggesting that T2D in Kuwait is probably caused by a complex interaction of genetic and environmental factors as described elsewhere [3,4].

An increased prevalence of glucose abnormalities and diabetes mellitus among patients with chronic hepatitis C virus (HCV) infection has been reported in several studies [5—10], including those done in Kuwait [11—13]. The connection between diabetes and HCV infection has also been demonstrated in anti-HCV seroprevalence studies in cohorts with diabetes. In a selected group of T2D-patients with abnormal serum aminotransferases, 28% of black and 12% of white patients had evidence of HCV infection [14]. A higher prevalence of HCV infection has also been reported in Spain in diabetic patients (11.5%) in comparison with blood donors (2.5%) [15]. Moreover, 4.2% of patients from a diabetes clinic in North America, were found to be positive for anti-HCV antibodies, compared with 1.6% in the control patients [7].

In spite of the growing number of reports showing a link between HCV infection and diabetes mellitus, the association of HCV infection with diabetes-related complications has not yet been clarified. In the current study, the prevalence of HCV infection in Kuwait population with T2D was first compared to that in Egyptian immigrants, owing to the fact that Kuwait nationals comprised only 32% of the population, and Egyptian immigrants comprised the major part of Arabs living in Kuwait [16]. The association of HCV infection with diabetes-related complications was then investigated.

Methods

Collection of blood samples

About 20% of the adult Kuwaiti population is estimated to have T2D [1,2]. The Kuwaiti adult population size is estimated to be 686,774 [16]. The sample size obtained by power calculation for simple random sample is 322 T2D-patients assuming 30% prevalence of HCV infection, 95% confidence interval and 0.05 as maximum accepted error. The inclusion criteria for this study were adult patients with documented T2D. The exclusion criteria were patients with other types of diabetes, diabetic patients with history of viral hepatitis, liver cirrhosis, or hepatocellular carcinoma. Venous whole blood samples in sterile 5 ml plain tubes were collected from 438 T2D patients (325 Kuwaitis and 113 Egyptians; 278 males and 160 females; median age, 47 years), recruited from Amiri Hospital, Mubarak Al-Kabeer Hospital, and Kuwait Diabetes Society. The HCV status was not known for any of the recruited diabetic patients at the time of patient visit and blood collection. Each patient included in this study was receiving one or combined medications according to his condition. The treatment included hypoglycemic drugs, antihypertensive drugs, and anti-cholesterol drugs. The last hemoglobin A1c (HbA1c) level and the presence of diabetes-related complications such as hypertension, hyperlipidemia, cardiovascular, renal, ocular or neurological disease, were recorded for all patients in addition to their demographic data including nationality, gender, age, body mass index (BMI), and the presence of family history of diabetes.

The same type of blood samples was also collected from 440 control subjects (308 Kuwaitis and 132 Egyptians; 255 males and 185 females; median age, 47 years) recruited from the same hospitals.
These control subjects included patients who came to the outpatient clinic of the hospital for follow-up after having received treatment for acute respiratory illness, acute gastroenteritis, or urinary tract infection. They had no chronic or malignant diseases. The HCV status was not known for any of the recruited control subjects. All the blood samples were collected after obtaining written informed consent from patients and controls. The ethical permission on this research study was granted by the Ethical Decision Committee of the Research Administration, Faculty of Medicine, Kuwait University, and by the Standing Committee for Coordination of Health and Medical Research, Ministry of Health of Kuwait.

Detection of HCV-specific antibodies by an immunnoassay (ELISA)

To determine the presence of HCV-specific antibodies in serum samples, the INNOTEST HCV Ab IV ELISA Kit (Innogenetics, Ghent, Belgium) was used according to the manufacturer’s instructions. The optical density (OD) values were determined at 450 nm by an ELISA reader, the Labsystems Multi Skan MS (Labsystems, Helsinki, Finland).

Nested-RT-PCR for the detection of HCV infection

Total RNA was extracted from the serum of patients and controls using the Qiagen QIAamp viral RNA mini kit (Qiagen Sciences, Maryland, USA). Eluted RNA was used for nested RT-PCR assay. The presence of HCV RNA from clinical specimens positive for anti-HCV antibodies was detected by nested RT-PCR assay as previously described [17]. The amplified PCR product was separated on 2% agarose gel electrophoresis, and visualized using UVP BioImaging System (UVP Ltd., Cambridge, UK). Image processing and analyses of DNA bands were performed using Labworks™ Image Acquisition and Analysis Software (UVP Ltd.).

Quantification of HCV RNA detected in T2D-patients and controls

Real-time reverse transcription-PCR for the quantification of HCV RNA was carried out on LightCycler 2.0 instrument, as described previously [18]. For absolute quantification, the pGEM-T Easy vector (Promega, Madison, USA) containing the 217-bp insert fragment of the 5′ untranslated region (UTR) of HCV genotype 4, was used at different concentrations to generate the standard curve. The linear range of the assay was from $5 \times 10^3$ to $5 \times 10^{10}$ copies/ml. Patients with an HCV viral load of more than 2 million copies/ml were considered as having high viral load.

HCV genotyping

The genotype of HCV RNA detected in clinical specimens was determined using the Versant HCV Amplification 2.0 LiPA assay (Siemens Medical Diagnostics Solutions, Tarrytown, NY, USA) according to the manufacturer’s instructions.

Statistical analysis

The continuous variables were expressed as median, and the two-tailed Mann–Whitney $U$-test was used to assess the difference between two groups. The $2 \times 2$ contingency table was performed for the categorical variables using the chi square ($\chi^2$)-test and Fischer’s exact test, as appropriate. Multivariate logistic regression analysis with backward stepwise selection method was used to evaluate the predictive baseline variables that could be associated with the presence of diabetes-related complications. All statistical analyses were performed using SPSS software version 17.0 for Windows (SPSS, Chicago, IL, USA).

Results

Hepatitis C prevalence in diabetic patients

The overall seroprevalence of HCV antibodies in T2D-patients (7%) was significantly higher than that in control subjects (1%) (OR = 8.3; 95% CI, 2.8—27.9; $p < 0.0001$). Kuwaiti and Egyptian T2D-patients have higher prevalence of HCV infection than that of their corresponding controls (Kuwaiti patients: $p = 0.001$; Egyptian patients: $p < 0.0001$). Egyptian T2D-patients were 6 times more likely to have HCV infection than Kuwaiti T2D-patients (OR = 6.1; 95% CI, 2.7—14.7, $p < 0.0001$, Table 1).

In the group of control subjects, 3 out of 4 HCV seropositive Egyptian subjects were HCV RNA positive, while in the group of T2D-patients, 24 out of 31 patients had evidence of HCV viremia (Table 1). The prevalence of HCV viremia in T2D-patients (5.5%) was significantly higher than that in control subjects (0.7%) (OR = 8.4; 95% CI, 2.5—44.05; $p < 0.0001$). HCV genotype 1 was detected in only two Kuwaiti patients, and all remaining HCV-infected patients and controls had HCV genotype 4 infection. The distribution of gender, family history of diabetes,
Table 1  
HCV infection in T2D-patients and controls.

<table>
<thead>
<tr>
<th>Subject group</th>
<th>HCV antibodies, n (%)</th>
<th>HCV RNA, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2D-patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kuwaitis (n = 325)</td>
<td>11 (3%)</td>
<td>8 (2.5%)</td>
</tr>
<tr>
<td>Egyptians (n = 113)</td>
<td>20 (18%)</td>
<td>16 (14%)</td>
</tr>
<tr>
<td>Males (n = 278)</td>
<td>21 (7.5%)</td>
<td>16 (6%)</td>
</tr>
<tr>
<td>Females (n = 160)</td>
<td>10 (6%)</td>
<td>8 (5%)</td>
</tr>
<tr>
<td>Control subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kuwaitis (n = 308)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Egyptians (n = 132)</td>
<td>4 (3%)</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>Males (n = 255)</td>
<td>4 (2%)</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>Females (n = 185)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Table 2  
Demographic data of T2D-patients with and without HCV infection.

<table>
<thead>
<tr>
<th>Gender</th>
<th>HCV antibodies, n (%)</th>
<th>HCV RNA, n (%)</th>
<th>p-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2D-patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kuwaitis</td>
<td>20 (64%)</td>
<td>11 (35%)</td>
<td>0.30</td>
</tr>
<tr>
<td>Egyptians</td>
<td>15 (59%)</td>
<td>10 (39%)</td>
<td>0.14</td>
</tr>
<tr>
<td>Males</td>
<td>16 (56%)</td>
<td>9 (35%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Females</td>
<td>4 (14%)</td>
<td>2 (8%)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Hepatitis C and diabetes-related complications

To investigate whether HCV viremia has a role in the occurrence or precipitation of diabetes-related complications such as hypertension, hyperlipidemia, cardiovascular, renal, ocular or neurological disease, the HbA1c levels and the proportion of T2D-patients with diabetes-related complications were determined in patients with and without HCV viremia. The HbA1c levels in T2D-patients with HCV viremia (median = 8.5%; range: 4.7—10.8) were significantly higher than those in HCV-negative patients (median = 6.7%; range: 4.5—10.8; p = 0.015). Diabetes-related complications were reported in 23 (96%) out of 24 T2D-patients with HCV viremia, and in 304 (73%) out of 414 T2D-patients with no evidence of HCV infection (p = 0.014). This association remained highly significant when the results were adjusted for other potential predictive variables for diabetes complications (Table 3). Female sex, age and family history of diabetes were also found to be independent risk factors for diabetes complications.

The association between HCV viremia and diabetes complications may be the result of a significant difference in the viral load between patients with and without diabetes complications. To test this hypothesis, HCV viral load was measured in all T2D-patients with HCV viremia. The median HCV RNA concentration in patients with diabetes complications was $4.1 \times 10^6$ copies/ml (range: $6.3 \times 10^4$ to $3.5 \times 10^7$ copies/ml), and that obtained in the single patient with no diabetes complications was $2.3 \times 10^5$ copies/ml.
Table 3 Independent risk factors for diabetes-related complications.

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>0.19 (0.09—0.38)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>1.07 (1.04—1.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Family history of diabetes (yes/no)</td>
<td>42.03 (15.83—111.63)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HCV viremia (yes/no)</td>
<td>23.65 (2.36—236.64)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Multivariate logistic regression model with backward stepwise selection method. The variables in each model included nationality, gender, age, body mass index, family history of diabetes, HbA1c levels and HCV viremia. OR, odds ratio; CI, confidence intervals.

5.5 × 10^5 copies/ml (p = 0.28). High viral load was only found in 12 (52%) out of 23 patients with diabetes complications.

Discussion

The high prevalence of HCV infection obtained in Kuwaiti T2D-patients (3%) compared to that in control subjects (0%), and to that previously reported in Kuwaiti blood donors (0.8%) [19], support previous studies that have noted a 2- to 10-fold increase in the seroprevalence of HCV infection in diabetic patients [7,14,20]. The higher prevalence of HCV infection in T2D patients may be due to increased exposure to medical interventions leading to an additional risk for acquiring HCV infection. However, this study cannot exclude the role of HCV infection in the development of diabetes, since results from previous studies have shown that patients with chronic HCV genotype 4 infection have an increased risk to develop glucose abnormalities [11—13].

The higher HCV seroprevalence in Egyptian T2D-patients compared to Kuwaiti patients could not be explained by a difference in the demographic data between the two populations. Even though the HCV seroprevalence in Egyptian patients with T2D was higher than that in control subjects (18% vs 3%), it was comparable to that described in the general Egyptian population [21]. Age, sex, marriage, rural residence, injections for bilharziasis, urography and blood transfusions have all been reported as risk factors for HCV infection in Egypt [21—23].

The results from this study have shown that HbA1c levels were not associated with diabetes complications. However, a single measurement of HbA1c levels can lead to an underestimation of the importance of HbA1c as a risk factor, as described earlier [24]. The updated mean of HbA1c using several values has been found to be better and is widely used [24—26]. Contrary to Hba1c results, HCV viremia was associated with increased occurrence of diabetes-related complications. Furthermore, the HCV viral load in 52% of patients with diabetes-related complications was high. These results should be confirmed with larger number of HCV-infected patients with and without diabetes-related complications.

Interestingly, the results showed that women have 5 times more risk to develop diabetes-related complications than men. The difference in patients’ gender with regard to the clinical presentation and clinical features of diabetes-related complications, has been well documented, with more significant impact on women than men [27—31]. The proportion of worldwide deaths attributable to diabetes mellitus is estimated to be higher in females than in males, with 1.5 million and 1.4 million deaths respectively [32].

Most of the HCV RNA detected in T2D-patients were of genotype 4. HCV genotype 4 is the predominant genotype in the Middle East including Kuwait [33,34]. Patients with genotype 4 have greater fibrosis progression rates, and are significantly more likely to have cirrhosis than are patients with different HCV genotype infections [35]. In addition, patients infected with HCV genotype 4 are less likely to respond to therapy than those infected with genotypes 2 and 3 [36,37]. A sustained virologic response rate of 68% to combination therapy has been reported in Kuwait in non-cirrhotic HCV-patients [38]. The overall prevalence of T2D in HCV genotype 4 infection in Kuwait was around 40% [12,13], which is not different from that reported in previous studies conducted in North America and Europe where different HCV genotypes are circulating [6,7,39]. The results of this indicate no difference between HCV genotypes detected in T2D patients and those detected in HCV-infected control subjects. In addition, there was no significant difference in the distribution of HCV genotype between patients with diabetes-related complications and those with no evidence of diabetes complications. Consequently, HCV genotype 4 is probably not associated with increased risk for diabetes-related complications. These observations are in line with results of an earlier study showing that HCV genotype 4 is not associated with increased risk for diabetes compared to other genotypes [11].

Conclusion

A higher prevalence of HCV viremia in patients with T2D compared to that in non-diabetic
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subjects, was found in this study. Since HCV viremia was associated with increased occurrence of diabetes complications, and since there is a growing body of evidence supporting the link between diabetes mellitus and the development of liver cancer in HCV-patients [40–42], national health promotion campaigns and prevention programs should be targeted to T2D-patients who are at high risk to contract a blood-borne infection like HCV.

Conflict of interest statement

All authors declare no conflicts of interest impeding the submission of this paper.

Acknowledgements

This work was supported by Kuwait Foundation for the Advancement of Sciences (KFAS) Grant No. 2006-1302-03, and by Kuwait University Research Grant No. MI 01/07.

References


