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Investigation of Insulin Receptor Substrate-1 and the Prevalence of Type 2 Diabetes Mellitus among Hepatitis C Infected Patients

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ABSTRACT

Background: *The hepatitis C virus causes a lot of deaths and illnesses across the world. It accounts more than 20% of hepatocellular carcinoma and chronic liver disease patients. In addition to causing liver illness, hepatitis C virus infection may produce extrahepatic symptoms, such as autoimmune diseases. Type 2 diabetes is more common in these individuals. There is strong evidence to support this theory, since research has shown that hepatitis C virus (HCV) infected individuals are more likely to develop type 2 diabetic mellitus (DM).* ***Aim of the study:*** *Estimation level of insulin receptor substrate-1 and prevalence DM in HCV patient* ***Material and Methods:*** *It is a case-control study that was held in Najaf governorate between July 2023 and December 2023 with a sample size of 128 subjects. The subject group included 128 (41 males and 23 females) HCV-infected patients, and the control group attended the specialized center for gastroenterology and hepatology and the public health laboratory. Their ages ranged from 26 to 70 years. Used ELISA Tanique for estimation of IRS-1, HCV IgG, and HbA1c and estimation of AST, ALT, and ALP in a spectrophotometer. The SPSS software package for Windows, version 27 (Build 27.0.1.0, copyright © IBM Corp., 2022, USA), was used to analyse the data.* ***Conclusion:*** *The hepatitis C virus leads to the destruction of IRS-1 in different ways and causes insulin resistance and type 2 diabetes mellitus.*

Keywords:Hepatitis C Virus, Diabetes Mellites, Insulin Receptor Substrate-1

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INTRODUCTION

The hepatitis C virus is easily identifiable by its diminutive size, which ranges from 55 to 65 nm in diameter. The entity may be viewed as a moving particle from time to time and has a spherical shape. Encased inside the virion's membrane is a positive-sense, single-stranded RNA virus. The genetic material of the virus is housed in a symmetrical nucleus that is 30-35 nm in diameter. A lipoprotein bilayer takes on the shape of an icosahedron and encases the core, providing protection [(1, 2).] The viral genetic code consists of a single nucleotide sequence that, when read in sequence, codes for 3,010 amino acids. At both the 5' and 3' ends of this sequence are non-translated regions (NTRs). The 5′-UTR IRES is the starting point for HCV protein translation, which results in a single polyprotein. Cellular proteases subsequently degrade this polyprotein, resulting in the structural proteins of the viral particles [(3)]. p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B are nonstructural proteins; core, E1, and E2 are structural proteins encoded by the 9.6-kb genome of the enveloped positive-strand RNA virus known as hepatitis C virus [(4)].

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Indirect routes of transmission, including contaminated materials, improper injections, and medical procedures, may also lead to the transmission of HCV [5]. People who inject drugs are at risk of contracting the HCV via the reuse and sharing of needles and syringes [6]. from a pregnant woman to her unborn kid and via sex transmission [7][8].The presence of chronic Hepatitis C infection has consistently been associated with other systemic illnesses, including diabetes mellitus and hepatic steatosis [9].

Hepatitis is the inflammation of the liver caused by certain viruses that specifically target and impact liver cells. These viruses may lead to both acute and chronic liver infections [10]. Rarely do people with acute infections acquire a diagnosis, and most of those who do don't exhibit any symptoms at all. The potential to spontaneously clear the virus without medical intervention occurs in about 30% (with a range of 15% to 45%) of infected persons within 6 months following infection. Chronic HCV infections may develop in around 70% of people, with estimates ranging from 55% to 85% [11]. A major health risk on a worldwide scale is chronic hepatitis C, which is an infection with the hepatitis C virus (HCV). Hepatocellular carcinoma, cirrhosis of the liver, and hepatocellular malignancy are all possible outcomes of hepatitis C virus infection [12].

The metabolic disorder known as diabetes mellitus is characterized by high blood sugar levels (hyperglycemia) due to an inadequate response to insulin or an absence of insulin production internally [13]. Characterized by the depletion of insulin-producing β cells in the pancreas, type I diabetes is a chronic immune system disorder [14]. Decreased insulin production and insulin resistance cause a relative shortage of insulin in type II diabetes, a medical disease [15]. There is substantial evidence between type II diabetes with chronic hepatitis C, according to many research [16]. percentages of IR and DM in HCV infected persons range from 2% to 9.4%, according to several studies; these percentages are lower than those in patients with other forms of chronic hepatitis [17].

Insulin receptors phosphorylate IRS, an intracellular cytoplasmic adaptor protein, on tyrosine residues. The IRS-1 is a vital family member that must be recognized. An increase in the quantity of high-affinity glucose transporter-4 (GLUT4) molecules on the outer membrane of insulin-responsive tissues, such as muscle cells and adipose tissue, occurs as a result of IRS-1 binding and phosphorylation. This causes these tissues to absorb more glucose from the bloodstream. The insulin signaling pathway is impaired in insulin resistance and diabetes mellitus (DM) due to abnormalities in the expression and activity of muscle IRS-1 [18].

Insulin resistance rises with the progression of age and liver fibrosis. It is suggested that HCV directly disrupts the body's glucose regulation since it is considerably raised during the early stages of chronic hepatitis C. Investigating the potential link between HCV and IRS-1 control has received a lot of attention [19]. Reduced efficiency of glucose absorption and utilization, together with anomalies in lipid metabolism, is the major cause of insulin resistance. Because insulin regulates glucose metabolism in the liver, skeletal muscle, and adipose tissue, insulin resistance develops in these tissues as pre-diabetes pathologically advances [20].

Insulin receptor substrate-1 (IRS-1) phosphorylation is the root cause of insulin resistance (IR), and the HCV core protein or other viral proteins may amplify this process. phosphatidylinositol 3-kinase (PI3K) activity is enhanced by phosphorylated IRS-1. The bulk of insulin's metabolic effects depend on PI3K activation and its downstream target, Akt. In this way, insulin resistance (IR) and the increased risk of diabetes in hepatitis C virus (HCV)

infections may be related to problems with the IRS-PI3K link or to the lack of PI3K activation [21].In addition to degrading insulin receptor substrates, the infection increased the expression of suppressor of cytokine signaling 3 (SOCS-3). caused the IRS-1 signaling molecule expression to drop [22].

MATERIAL AND METHODS

Study design and sample collection

It is a case-control study that was held in Najaf governorate between July 2023 and December 2023 with a sample size of 128 subjects. The subject group included 128 (41 males and 23 females) HCV-infected patients, and the control group attended the specialized center for gastroenterology and hepatology and the public health laboratory. The age range was 26–70 years, and patients were enrolled after a diagnosis of HCV by a professional physician.

The questionnaire included name, age, and history of other hepatitis infection for the patient group, patients with other autoimmune diseases, and in the case of the control group, any diabetic or hepatitis virus infection. The patients were aware of the study, and verbal approval had been obtained with the criteria of inclusion: hepatitis C virus seropositive patients with and without type 2 diabetes mellitus for the patient group, and diabetics and HCV for the control group. The exclusion criteria include cardiovascular disease patients, gestational diabetic females, people with incomplete data, patients with autoimmune diseases, and other types of hepatitis viruses.

Gel tubes were used to collect 5 ml of blood samples from 128 patients diagnosed with hepatitis C virus infection, as well as from the control group. The whole blood sample was left to coagulate at room temperature for a minimum of 10 minutes, after which it was circulated in a centrifuge for 5–10 minutes at a speed of 3000 RPM. The serum was separated into five Eppendorf tubes, each containing 0.5 mL. Four of these tubes were kept at a temperature of -80°C in the deep freezer for future use when sample collection was finished. The remaining tube was submitted for common liver function testing.

The enzyme-linked immune sorbent assay (ELISA) kit (Sunlong Medical Company, China) is a is a technique that depends on a colorimetric approach to generate quantifiable data used for HbA1c and IRS-1, and the ELISA kit (DALAB, Austria) for HCV IgG. The level of enzymes in the liver function test (AST, ALT, and ALP) by Nipigon/Canada.

Ethical approval

This study takes the ethical approval from the internal ethical committee of the medical microbiology department/faculty of medicine / university of Kufa and the health directorate in AL-Najaf AL-Ashraf province by all of the included patients or their parent's prior participation.

STATISTICAL ANALYSIS

The SPSS software package for Windows, version 27(Build 27.0.1.0, copyright © IBM Corp, 2022, USA) was used to analyze the data for the results, Descriptive statistics were used initially to show all results were data are presented as the mean± standard deviation (SD). An independent sample test was used to examine the comparison between patients and healthy groups, namely the male and female groups. The analysis one-way (ANOVA) was used to compare the age groups of patients and get the least significant difference (L.S.D). Pearson's correlation coefficients(r) were used to determine the correlation between parameters. P-value <0.05 was considered significant.

RESULTS

This study involves 128 subjects, divided into two groups: 64 HCV-infected patients and 64 healthy subjects. Figure 1 shows a significant decrease ($p<0.05$) in the in the serum concentration of insulin receptor substrate 1 in the patient group (341.8593±254.21752) as compared with the control group (511.1744±136.72896).

Table No. 1 showed a significant difference in HbA1c in patients (336.841± 144.6335) as compared with Results in Table No. 2 show a significant increase in liver enzymes (AST, ALT, and ALP) in the patient group as compared to the control group (p-value <0.05). controls (274.858± 30.2291) in p-value <0.05.

In figure No. 2, explain that the result of IRS-1 in the serum of the male patient group has significantly increased ($p<0.05$) as shown in Table 4.2.1 (382.5790± 249.04513) rather than in the serum of the female group patient (269.2720± 252.38557).

Table No. 3 showed that males significantly increased in these parameters (HbA1c, AST, ALT, and ALP) than females (p-value <0.05).

The result of IRS-1 in figure 3 explain the compression between group patient in according to age reveal the age group (51-60) have the high percent in about (454.21±238.74113) with age group (61-70) in about (414.16±216.40564) in gradually other age group (31-40) in about (385.40±249.47753) and (41-50) in about (299.94±284.52592) and (26-30) in about(188.22±235.14609).

Figure No. 4 shows that the serum concentration of IRS-1 correlated positively and significantly with HbA1C patients (R2 = 0.636).

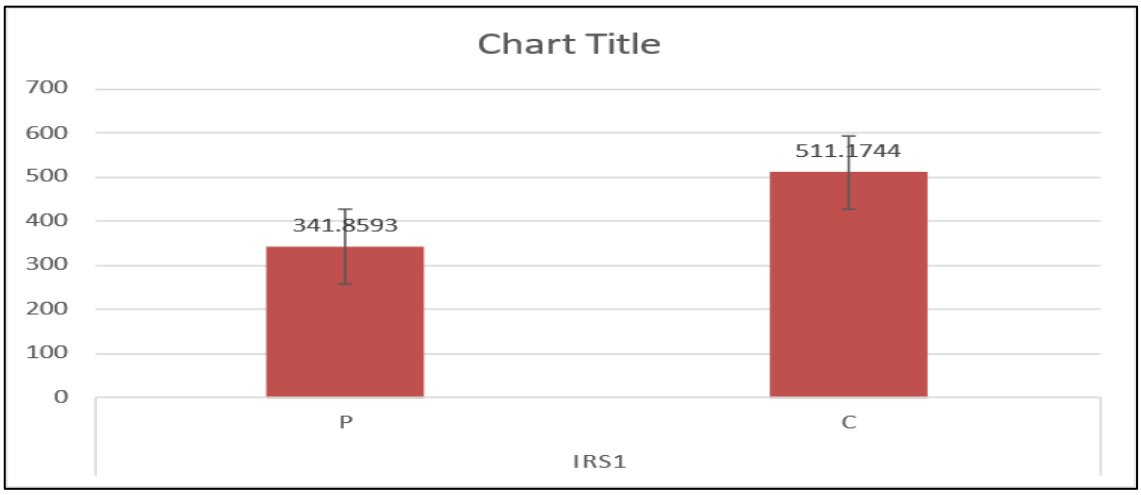

Figure No.: Compression serum level of IRS-1 in patient and control group.

Table No. 1: Comparison of IRS-1 and HbA1C between patient and control.

Markers of study	Patients with HCV n=64	Control without HCV n=64	p-value
IRS-1 pg/ml Mean ± SD	341.8593±254.21752	511.1744±136.72896	0.0001
HbA1C % Mean ± SD	336.841± 144.6335	274.858± 30.2291	0.0001

(SD= standard division, P value=probability value (level of significance at <0.05)

Table No.2: Comparison some liver function enzyme AST, ALT, ALP between patient and control.

Some liver enzyme of study	Patients with HCV n=64	Control without HCV n=64	p-value
AST mg/dl Mean ± SD	30.266± 14.0118	22.310± 9.0885	0.0001
ALT mg/dl Mean ± SD	21.939±8.9219	9.630±6.1614	0.0001
ALP mg/dl Mean ± SD	271.745± 130.5765	22.310± 39.0589	0.0001

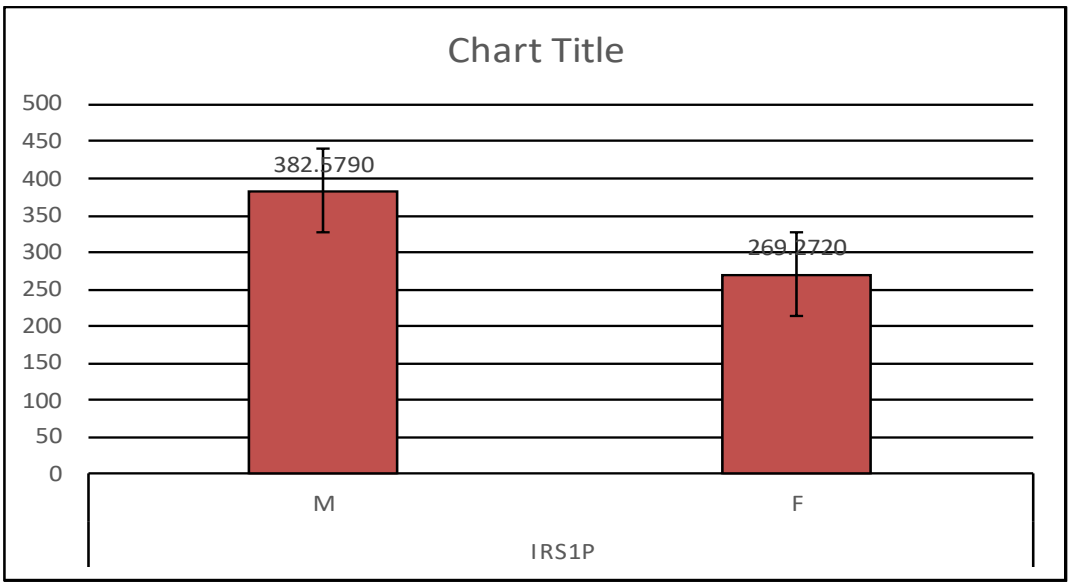

Figure No.2: Compression of IRS-1 in patient group according to sex.

Table No.3: Compression between male and female HCV group patient.

Parameter of study	Male	Female	P -value
HbA1c Mean ± SD	351.982± 150.5001	309.850± 132.3498	0.001
AST Mean ± SD	32.770± 14.3200	28.586± 13.2689	0.011
ALT Mean ± SD	16.772± 10.3088	15.375± 12.1917	0.034
ALP Mean ± SD	275.661± 110.9519	267.725± 162.4273	0.021

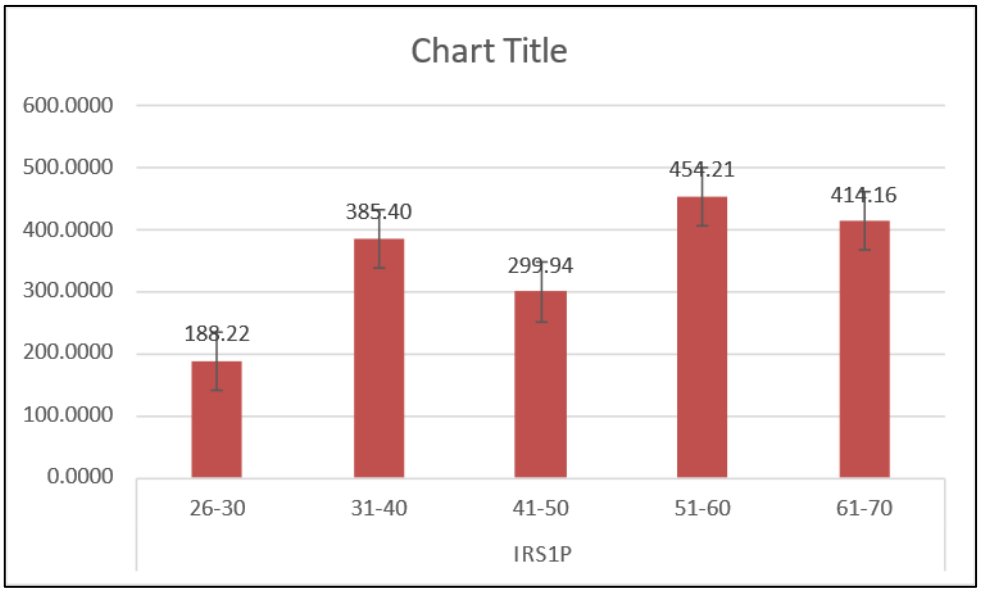

Figure No. 3: Age group patient of IRS-1.

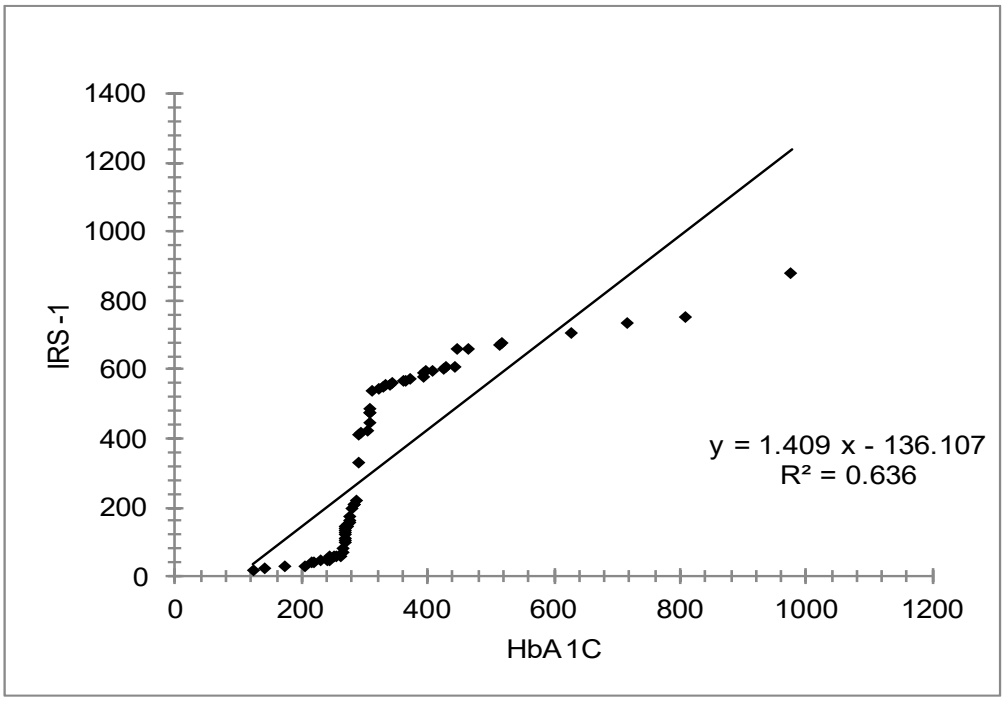

Figure No. 4: Correlation of IRS-1 with HbA1C patients.

DISCUSSION

Globally, the hepatitis C virus is one of the leading causes of illness and death. Around 25% of occurrences of hepatocellular carcinoma and chronic liver disease are caused by it. There are a number of extra-hepatic symptoms associated with HCV, including Type 2 Diabetes Mellitus (T2DM) (23), but the virus mostly affects the liver (24). The effects of diabetes mellitus on people all around the world are substantial. Evidence suggests that people with hepatitis C are more likely to develop type 2 diabetes mellitus. The idea that HCV infection increases the likelihood of developing TIIDM has been backed by several research and pieces of evidence (25). Similar to the findings reported by Kawaguchi et al. (2004), who found that the expression levels of IRS1 and IRS2 decrease in HCV-infected patients as hepatic fibrosis advances, figure No. 1 shows that insulin receptor substrate 1 (IRS-1) serum levels are significantly lower in HCV-infected patients

compared to a control group. These results show that hepatitis C virus (HCV) causes insulin resistance by changing specific liver components that regulate glucose metabolism. Inhibition of SOCS3 synthesis by the HCV core protein may improve proteasome-mediated IRS1 and IRS2 degradation (26). (Parvaiz, F., *et al*. 2011)these result of study same to the current study that report the structural and non-structural proteins of HCV disrupt cellular gene expression, impairing insulin signaling and thereby causing diabetes mellitus [27].

Table No. 1 shows that patients had higher HbA1c levels compared to the control group. This is in line with the findings of Abdelkader, R. Y., et al. (2021), who also found a significant association (P 0.05) between blood glucose levels, biological parameters linked to liver disease, and HCV-Ab concentration. Research has shown that people with chronic hepatitis C (CHC) are more likely to develop fibrosis when their glucose levels are high (28). In table No. 1 demonstrate increase HbA1c in patient than control these same to Abdelkader, R. Y., *et al*. (2021) which also reported a significant correlation ($P < 0.05$) between blood glucose levels and liver disease-related biological parameters and HCV-Ab titer. These findings demonstrate that increased glucose levels have a fibro genic effect on individuals with chronic hepatitis C (CHC) [28]. The present research found a rise in liver enzymes (AST, ALT, ALP) in table No. 2. This finding is consistent with Jain et al. (2023), which also found an increase in these parameters in infected individuals [29]. Additionally, our findings are in line with those of Anand, B. S., and M. Velez (2004), who reported that the viral load of HCV had no effect on the severity of liver disease or the elevation of liver enzymes. A complicated interaction between the virus and the host's immune response is thought to determine the severity of liver damage [30].

Consistent with the research done by Crisan et al. in 2017, the present result of figure two of IRS-1 indicates that insulin resistance may be more common in females with HCV infection compared to men. Insulin resistance is more common in females with HCV compared to males, and this remains true even after controlling for other factors [31]. Consistent with (Javed, M. J. M., et al. 2013), which analysed data from 200 patients and found that 68 (or 34% of the total) had impaired glucose tolerance, table No. 3 of the present research demonstrated that males infected with HCV had higher HbA1c levels than females. There were 43 men and 25 women, or 63% and 36% respectively. All of the patients had impaired glucose tolerance due to cirrhosis after obtaining hepatitis C virus. Impaired glucose tolerance was much more common among patients with decompensated cirrhosis due to hepatitis C, according to the study's major result [32].

Additionally, our findings corroborate those of (Smith et al., 2024) which found that male HCV patients had greater levels of liver enzymes than female patients, as shown in table No.3 of the present study. Hepatocellular carcinoma was also more common in males. Metabolic risk factors were only weakly associated with males, which led to worsening liver disease and less access to effective therapies [33].

Figure No. 3 displays the results of the present investigation broken down by age group. It should be noted that there is a dearth of focused study on this specific subject. We conclude that hepatitis C virus infection may cause fibrosis and cirrhosis in the liver, which in turn may impact insulin signaling pathways negatively. The insulin signaling and glucose metabolism pathways in the liver are mediated by IRS-1. It is possible that IRS-1 expression or activity is influenced by the degree of liver disease in older individuals with HCV. Figure 4 shows the positive and statistically significant association between IRS-1 and patients' HbA1C levels in this research. Results from the study by (Mahmutovic et al. in 2019) corroborate these

findings. Furthermore, the data demonstrated a correlation between the rs7578326 SNP in IRS-1 and elevated HbA1c levels, adding to the mounting evidence that this genetic variation is involved in glucose control [34].

CONCLUSION

The current study demonstrated that:

1-Reduction in IRS-1 levels in individuals infected with HCV.

2- Patients infected with HCV have elevated liver enzymes along with unregulated HbA1c levels.In the group infected with HCV,

3- male patients show a greater rise in HbA1c and liver enzyme levels compared to female patients.

4-In HCV, a reduction in IRS-1 has a greater impact on females compared to men.

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