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Investigation of Interleukin-22 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 for almost 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 diabetes mellitus (DM).* ***Aim of the study****: Estimation of IL-22 and the prevalence of DM in HCV patients.* ***Material and Methods:*** *It is a case-control study that was held in the 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. We used ELISA Tanique for the estimation of IL-22, HCV IgG, and HbA1c and the 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 analyze the data.* ***Conclusion:*** *The hepatitis C virus led to an increase in IL-22 in HCV-infected patients and uncontrolled HbA1c with an increase in liver enzyme, and males were more likely to increase IL-22 than females.*

Keywords: Hepatitis C virus, Diabetes Mellites.

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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 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]. Other systemic diseases, such as diabetes mellitus and hepatic steatosis, have repeatedly been linked to chronic Hepatitis C infection [9]. The inflammation of the liver brought on by viruses that prey on liver cells is known as hepatitis. Acute and chronic liver infections are both caused by these viruses [10].

Rarely do people with acute infections acquire a diagnosis and most of those who 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].

One of the cytokines that belongs to the Interleukin -10 family is Interleukin-22. Other members of this family are Interleukin-10, 19, 20, 24, 26, 28α, 28α, and 29. To make it, cells from the adaptive and innate immune systems, such as αζ T cells, γδ T cells, NKT cells, and innate lymphoid cells (ILCs), combine. The IL22 gene is located on chromosome 12q15, near the IL26 locus and 99 kbp upstream of the IFNG locus, respectively. When it comes to regulating immune cell activity, IL-22 is unique among cytokines. Rather, it mostly attacks cells located on the body's outside, including those in the skin, digestive and respiratory tracts, the pancreas, the liver, the kidneys, and the joints [18].

Increased IL-22 expression in the liver is positively associated with the degree of liver fibrosis in individuals infected with hepatitis C virus (HCV). A protective strategy to prevent liver damage, however, IL-22 positive cells accumulate more prominently in patients with greater degrees of fibrosis [18]. According to research, IL-22 may protect the liver from damage induced by CCL4 and FAS ligand (FasL). Moreover, a substantial body of research suggests that IL-22 plays a key function in promoting the growth of liver tumor cells, which may amplify their detrimental impacts. New evidence suggests that IL-22 is upregulated in HBV and HCV infected individuals and animals, exacerbating chronic inflammation and fibrosis in the liver. The local cytokine milieu, in addition to the specific characteristics and location of the

afflicted tissues, dictate the extent to which these cytokines contribute to a variety of human disorders. the year [19].

Reduced IL-22 plasma levels were seen in individuals with impaired fasting glucose (IFG) and type II diabetes mellitus. The decline in IL-22 is associated with an uptick in type 2 diabetes mellitus and impaired fasting glucose (IFG). Further research into the mechanisms behind the relationship between IL-22 levels, insulin resistance, type II diabetes mellitus (DM), and the general population's susceptibility to developing diabetes is needed. Recent research suggests that IL-22 may influence the metabolically important JAK-STAT3 pathway, however the precise effect of IL-22 on metabolism is yet unclear. Based on these results, IL-22 could be involved in the development of metabolic syndrome and type II diabetes [20].

MATERIAL AND METHODS

Study design and sample collection

It is a case-control study that was held in Najaf governorate between July 2023 to December 2023 in a sample size 128 subjects. The subject group included 128 (41 males and 23 females) HCV-infected patients (41 males and 23 females) who control group attended to a specialized center for Gastroenterology and Hepatology, and the public health laboratory the age ranged (26-70 yrs.) and patients were enrolled after a diagnosis of HCV by the professional physician. The questionnaire included name, Age, and history of other hepatitis infections for the patient group, patients with other autoimmune disease, 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 criteria of inclusion, Hepatitis C virus seropositive patients with and without type 2 diabetes mellitus for the patient group and should be without diabetes 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 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. Enzyme-linked immune sorbent assay (ELISA) kit (sunlong Medical Company, China) technique that depends on colorimetric approach to generate quantifiable data used for HbA1c and IRS-1 and ELISA kit (DALAB /Austria) for HCV IgG. The level of enzyme liver function test (AST, ALT, 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. In **Fig. 1,** there was a significant increase ($p<0.05$) in the serum concentration of interleukin 22 in the patient group (HCV-infected patient) (17.9195 ± 7.89684) as compared with the control group (normal patient) (13.4714 ± 2.55074). Table No. 1 showed a significant increase in HbA1c in the patient (334.8413± 142.63354) as compared with the control (272.8589± 29.22914). 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).

In Fig. No. 2, explain the result of IL-22 in the serum of the male patient group ($p<0.05$) (19.7422± 9.19560) rather than in the serum of the female group patient (14.6704± 2.75776).

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 IL-22 in **Fig. No. 3** explains the compression between group patients according to age, revealing that the age group (61-70) has a high percent in about (20.25±8.49714), with the age group (26-30) in about (19.38±10.60073) in gradually other age groups (31-40) in about (17.97±7.43638), (51-60) in about (15.05±3.91130), and (41-50) in about 14.14±2.65477.

Figure No. 4 shows that the serum concentration of IL-22 correlated positively and significantly with HbA1C patients (R2 = 0.627).

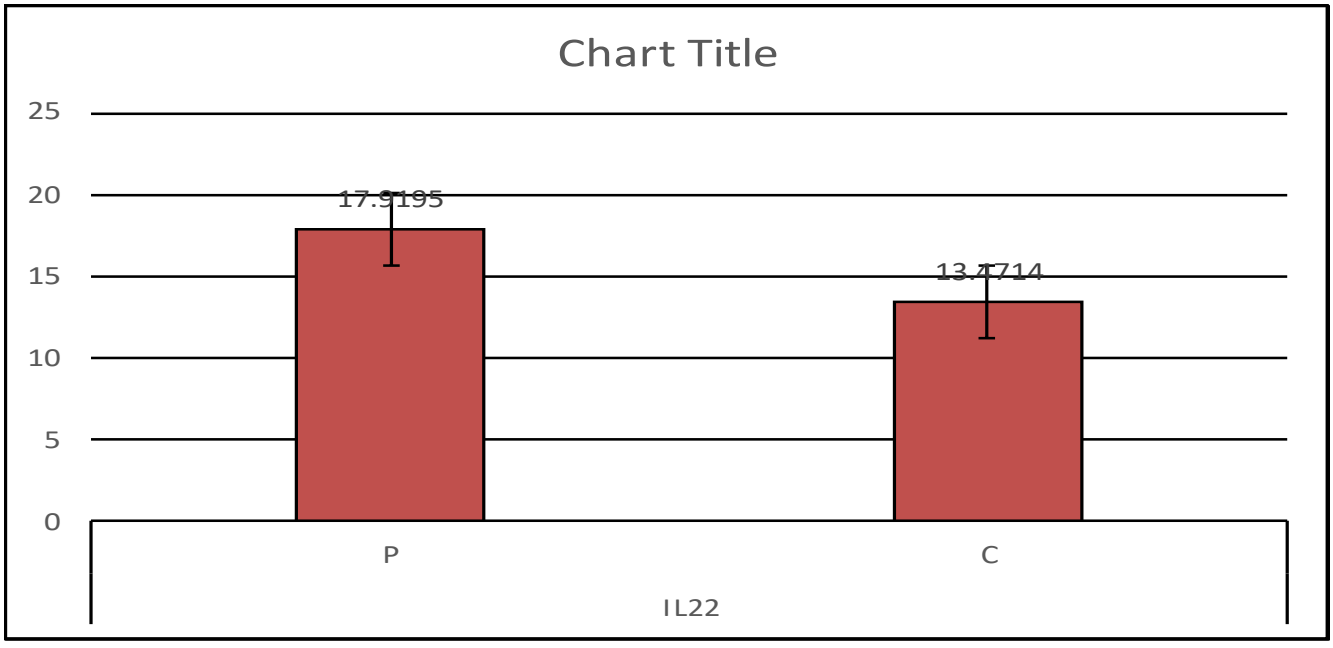

Figure No. 1: compression serum level of IL-22 in patient and control group.

Table No. 1. Compression of IL-22 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	334.8413± 142.63354	272.8589± 29.22914	0.0001

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

Table No.2 Comparison some liver function enzymes 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	29.2664± 13.01186	21.3105± 8.08857	0.0001
ALT mg/dl Mean ± SD	20.9394±7.92198	8.6300±5.16145	0.0001
ALP mg/dl Mean ± SD	270.7455± 129.57654	21.3105± 38.05899	0.0001

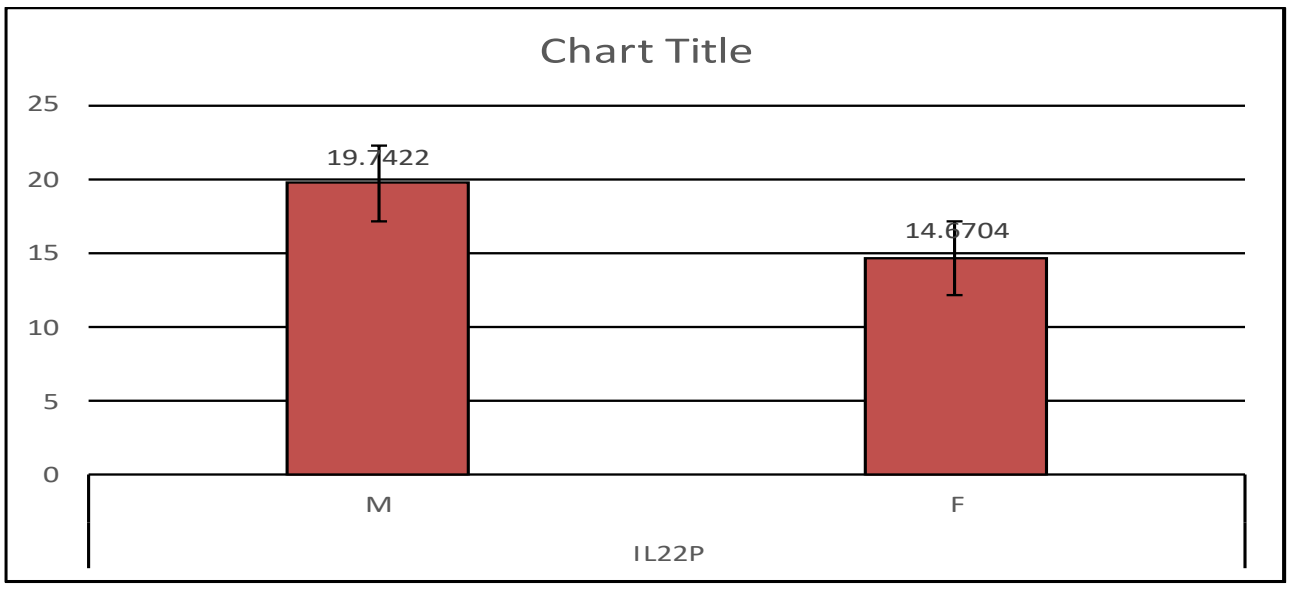

Figure No.2 Compression of IL-22 in a patient group according to sex.

Table No.3 Compression between male and female HCV group patients.

Parameter of Study	Males	Females (Mean ± SD)	P -value
HbA1c	350.9822± 149.50015	308.8509± 131.34985	0.001
AST	31.7700± 13.32000	27.5861± 12.26896	0.011
ALT	15.7727± 9.30888	14.3757± 11.19176	0.034
ALP	274.6617± 109.95191	266.7252± 161.42734	0.021

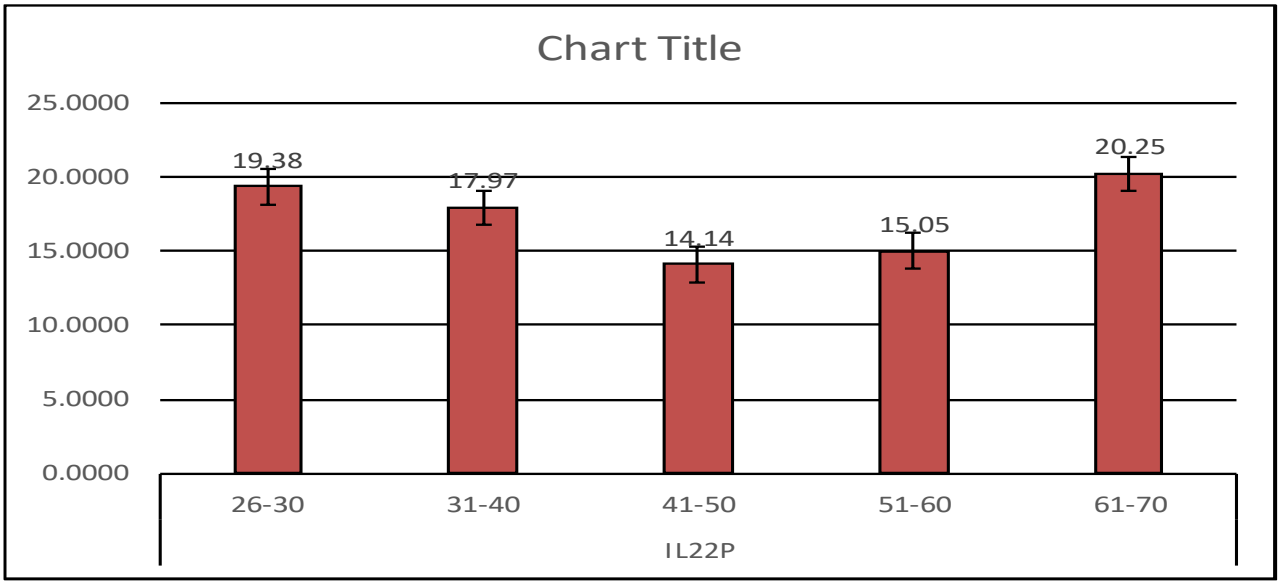

Figure No. 3 Comparing between age groups for IL-22.

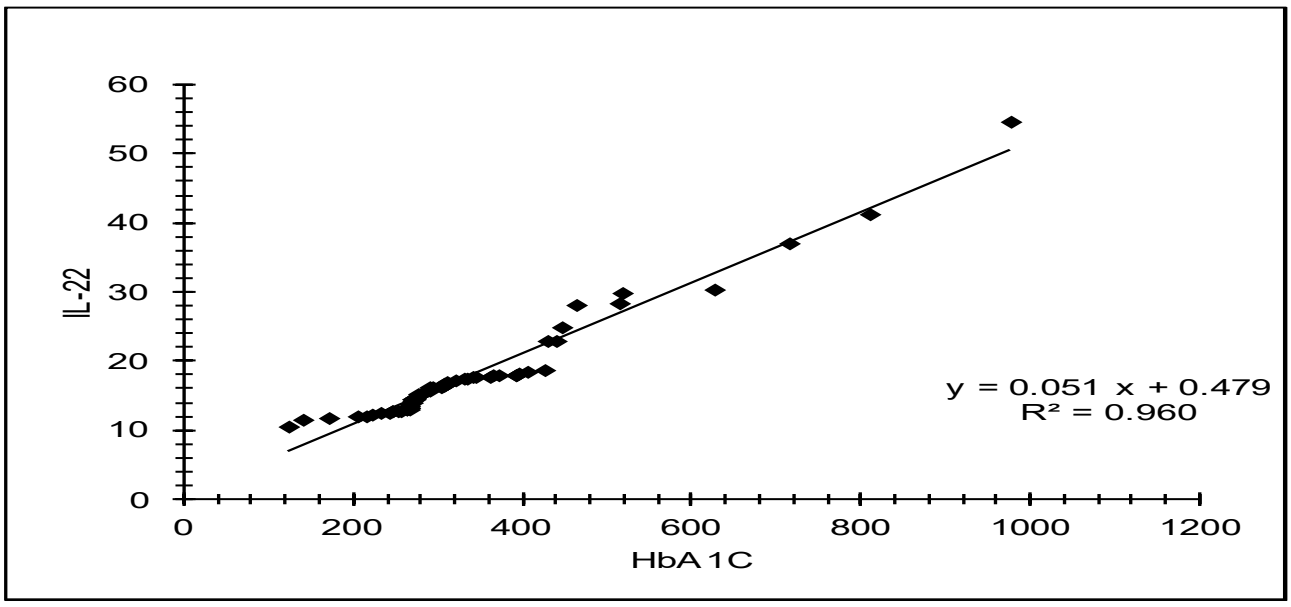

Figure No. 4 Correlation of IL-22 with HbA1C patients.

DISCUSSION

One of the leading causes of illness and death on a global scale is the hepatitis C virus. About a quarter of all instances of hepatocellular carcinoma and chronic liver disease have this cause. Type 2 diabetes mellitus (T2DM) is one of the extra-hepatic symptoms that have been associated with the hepatitis C virus (HCV), while the virus mostly damages the liver (21)(22). 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. There is substantial evidence from many research that indicates that having an HCV infection increases the likelihood of developing TIIDM (23). Compared to controls, HCV patients had greater levels of IL-22, as shown in Figure No. 1 of the present research. The findings are consistent with those of (GM, F., *et al*. 2017), who also found that levels rose sharply in response to antiviral therapy [(24)]. The buildup of IL-22 in the liver tissues was greater in patients with chronic hepatitis C infection, according to (Park, O., et al. 2011), but it was much more apparent and more widely infiltrated in patients with liver cancer [(25)]. According to (Foster et al. 2012), the frequency of CD4 T cells generating IL-22 does not alter significantly depending on the infection outcome or when compared to studies without a control participant [(26)]. According to a 2014 study by Pan et al., IL-22 is ineffective against HCV replication compared to IFN-a or another kind of interferon. Furthermore, IL-22's direct effects on antiviral protein gene transcription remain poorly supported [(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)]. The present investigation found that liver enzymes (AST, ALT, ALP) increased in infected individuals compared to controls [(29)], which is in line with the findings of Jain et al. (2023). 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 (Resham, S., et al. 2020), who failed to find any variations in IL-22 levels between men and females, the present study's Figure No. 2 shows that IL-22 concentrations are higher in the male-infected group than in the female-infected group. For example, individuals with HCV were more likely to develop cirrhosis and hepatocellular carcinoma, according to the study. In addition, males had a greater incidence of these disorders, which, when measured in patients, may indicate an increased IL-22 concentration [(31)]. Consistent with (Javed, M. J. M., et al. 2013), who analyzed data from 200 patients and found that 34% 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 the 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)].

Table No. 3 of the present study confirms that male HCV patients exhibited greater enzyme levels than female patients, which is in agreement with the findings of (Smith et al. 2024) and our own findings. Furthermore, males had a higher incidence of hepatocellular carcinoma. More severe liver disease progressed and effective therapies were harder to come by because of the poor correlation between metabolic risk variables and men's gender [(33)]. The age group 61-70 has the biggest proportion, followed by the age group (26-30), with the remaining age groups having lesser percentages, according to the present study's results according to age in Figure No. 3. Consequently, the fluctuation of IL-22 levels across various age groups has not been the subject of any more focused inquiry as of yet. In our view, those over the age of 60 may be more susceptible to viral-induced alterations in IL-22 levels, which in turn may lead to autoimmune hepatitis. Several factors may play a role in this, such as the use of immunostimulant medicine, the success or failure of infection treatments, or the existence of underlying genetic or immunological disorders.

The chance of becoming ill among the remainder of the age group is greatly affected by the patient's immunological condition, particularly in those between the ages of 26 and 30. Immunological activity is frequently highest in this age range. There is a correlation between the increased IL-22 level in HCV-infected people and the genetic variables that influence both the IL-22 level and viral load. As seen in Figure No.4, the current investigation discovered a favorable association between IL-22 and HbA1c. These results are in line with those of the study by Guo et al. 2016), which, after comparing patients' HbA1c levels to those of the control group, likewise found an association between the presence of T2DM and IL-22. Type 2 diabetes mellitus and other diseases affecting the big and small blood vessels all share insulin resistance as a hallmark. Importantly, they found that BMI and IR were significantly correlated with the frequency of Th22 cells and IL22+IL-17+CD4+ T cells [(34)].

CONCLUSION

The current study demonstrated that:

1-people infected with HCV have elevated levels of IL-22 compared to healthy persons.

2-The liver enzyme and HbA1c levels were found to be higher in HCV patients compared to healthy persons in this investigation.

3- Males infecte d with HCV have a higher IL-22 level compared to females.

4- Males infected with HCV have a greater rise in HbA1c levels and liver enzyme levels compared to females.

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