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Comparative Study of SIRT1 Expression in Hepatitis B Virus, Hepatitis C Virus, and Hepatocellular Carcinoma Patients

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Abstract:

Background and Aim: The most common form of primary liver cancer in adults is hepatocellular carcinoma (HCC), and it is the most common cause of death in people with cirrhosis. The mammalian Sir2 family or sirtuins is formed of a cluster of highly conserved proteins triggering nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylase activity which target histone and non-histone substrates including enzymes, transcription regulators, tumor suppressors, cell signaling proteins and DNA repair proteins. Seven human sirtuins have been identified and named SIRT1-7. SIRT1 has been regarded as a tumor promoter due to its increased expression in some types of cancers and its role in the inactivation of proteins involved in tumor suppression and DNA damage repair. We aimed to estimate the level of SIRT-1 in the blood of HBV, HCV, and HCC patients, to investigate the possible role of SIRT-1 in the pathogenesis of HCC and to determine the role of SIRT-1 as a tumor marker or pomoter compared to other markers like α-feto protein. **Subjects and Methods:** This study was performed in Internal Medicine department Beni-Suef Hospital. Our study included 160 individuals; divided into four groups; group A (healthy control), group B (HBV patients), group C (HCV patients) and group D (HCC patients). The serum level of SIRT-1 was assessed and compared in all groups and correlate its level as a tumor marker with the other marker α-fetoprotein. **Results:** There was significant increase in SIRT-1 levels in HBV, HCV and HCC group as compared to control (p value <0.001). There was significant increase in SIRT-1 in HCV and HCC groups as compared to HBV group (p value <0.001). There was significant increase in SIRT-1 levels in HCC group as compared to HCV group (p value <0.001). There was positive correlation between α - feto protein and SIRT-1in HCC group only but no correlation in others groups. **Conclusion:** Our study demonstrated that SIRT-1 is overexpressed in HCC patients compared to

chronic noncancerous viral liver parenchyma diseases, so it may be included in pathogenesis of HCC. SIRT-1 may have matchable specificity and sensitivity as AFP in HCC patients.

Keywords: HCC; HBV; HCV; SIRT-1

1. Introduction:

Hepatocellular carcinoma (HCC) is the most common primary liver cancer in adults is, and it is the most common cause of mortality in cirrhotic patients. It occurs in the form of chronic inflammation of the liver and is most closely associated with chronic infection with viral hepatitis (hepatitis B or C) or with exposure to toxins such as alcohol or aflatoxin. The risk of developing HCC is significantly increased by some disorders, such as hemochromatosis and alpha 1-antitrypsin deficiency. NASH and Metabolic syndrome are also increasingly recognized as risk factors for HCC [1].

A cluster of highly conserved proteins that activate nicotinamide adenine dinucleotide (NAD)-dependent activity of histone deacetylase that target histone and non-histone substrates including enzymes, transcription regulators, tumour suppressors, cell signaling proteins, and DNA repair proteins are the mammalian Sir2 family or sirtuins [2].

There have been seven human sirtuins described and named SIRT1-7. The NAD+-dependent catalytic core domain of all the family members' functions as NAD+-dependent deacetylase (DAC) and/or mono-ADP-ribosyl transferase [3].

The human sirtuin, SIRT1, is the most extensively researched. It has potential effects on cell processes ranging from cell survival through its substrates to apoptotic signaling. It also deacetylates many other proteins and helps to control the cellular pathways involved in stress reactions [2].

SIRT1 has been considered a tumour promoter because of increasing expression in some forms of tumours and its function in inactivating proteins involved in tumour suppression and repair of DNA damage, [4].

So, aiming the molecular mechanism of tumor promotion early may help for better prognosis. Multiple studies demonstrated that there is a significant association between HCC promotion and SIRT1 overexpression, Thus, encounter of its overexpression may have a therapeutic role in controlling HCC [4].

2. Patients and Methods:

This study was conducted at Beni-Suef University hospital, Beni-Suef Egypt within six months from November 2018 to May 2019, involving 160 patients. The protocol of study was approved by Ethical Committee of Beni-Suef Faculty of Medicine. Informed consents were obtained from participants.

2.1 Inclusion criteria:

- Age between 18 to 70.
- Patients with HBV or HCV or Hepatocellular carcinoma

Exclusion criteria:

- Age below 18 and above 70.
- Other types of liver diseases.
- Patients with other cancers.
- Received any specific treatment for HBV or HCV or HCC.
- Chronic kidney disease.

The 160 studied subjects were divided into four groups as follows:

- Group A: (n = 40) Healthy Control.
- Group B: (n = 40) HBV patients.
- Group C: (n = 40) HCV patients.
- Group D: (n = 40) HCC patients.

All the patients were subjected to full history taking, clinical examination and laboratory and radiological investigations including complete blood count, prothrombin time and concentration, serum albumin, total bilirubin, Alkaline phosphatase, serum creatinine, alanine transaminase (ALT), aspartate transaminase (AST), Hepatitis B surface antigen (HBsAg), HCV antibody and HCV RNA, alpha fetoprotein (AFP), abdominal examination and abdominal CT. HCC was diagnosed on the basis of at least two imaging methods (ultrasound, CT) and biochemistry (alpha fetoprotein).

C. Estimation of serum levels of Sirt-1

Blood samples were collected from all subjects. Serum was separated from blood sample and stored at –80°C to be used for detecting the level of Sirt-1 by ELISA. Sirt-1 was detected by Human Sirtuin 1 (SIRT1) ELISA kit (Catalog Number MBS2601311).

Statistical methodology:

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 24. Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests (Chan, 2003a). Correlations between quantitative variables were done using Spearman correlation coefficient (Chan, 2003b). P-values less than 0.05 were considered as statistically significant.

3. Results:

The present study was a comparative study that included 160 Egyptian individuals whose age ranged from 18 to 70 years. They were recruited from Internal Medicine outpatient clinic at Beni-Suef University Hospital.

The 160 studied subjects were divided into four groups as follows:

- Group A: (n = 40) Healthy Control.

- Group B: (n = 40) HBV patients.
- Group C: (n = 40) HCV patients.
- Group D: (n = 40) HCC patients.

In all the following tables and figures; Expression of data is as Mean ± SD, p value <0.05 was significant

(a) Denotes significant difference versus normal control group.

(b) Denotes significant difference versus HBV group.

(c) Denotes significant difference versus HCV group.

Table (1): Comparison between The studied groups as regard to Demographic and routine laboratories

Demographic and Laboratory Data	Normal Group (A)	HBV Group (B)	HCV Group (C)	HCC Group (D)
Age	45.25±9.27	42±9.27	39.5±21.92	55±7.83 abc
Sex (Male %)	80%	65%	70%	95% abc
AST	21.75±7.54	56±27.96 a	39.9±21.3 ab	64.15±38.91 ac
ALT	21.5±8.24	54.33±30.83 a	53.04±27.88 a	56.45±28.26 a
Total Bilirubin	0.81±0.34	0.91±0.28	0.74±0.39	2.07±1.48 ac
Direct Bilirubin	0.26±0.16	0.25±0.06	0.24±0.15	1.1±0.96 ac
Alkaline Phosphatase	76.65±27.43	100±22.07 ac	66.77±37.57	94.55±24.92 ac
Albumin	4.56±0.65	4.2±0.93	4.11±2.11 a	3.36±0.67 abc
Creatinine	0.65±0.30	0.90±0.20	0.92±0.52 a	0.97±0.33 a

Table (1) show there is significant difference between HCC group and all other groups as regard Age, Sex and Albumin while there is significant difference between HCC group and both groups (A: control and C: hepatitis C as regard AST, Total and direct bilirubin and Alkaline phosphatase. HCC group (group D) has significant difference with control group only as regard ALT and creatinine. Group C (HCV) patients had significant difference compared with control group (group A) as regard Albumin, AST, ALT and creatinine. Group B (HBV) patients had significant difference compared with control group

(group A) as AST, ALT and Alkaline phosphatase. There is significant difference between group B(HBV) and group C (HCV) as regard AST and Alkaline phosphtase.

Other Markers:

1. **Hepatitis B surface antigen (HBsAg):**

In HCC group, two (5%) patients were HBsAg positive and thirty-eight (95%) patients were HBsAg negative.

2. **HCV Antibody and HCV RNA:**

In HCC group, thirty-eight (95%) patients were HCV Antibody and RNA positive and two (5%) patients were HCV Antibody and RNA negative.

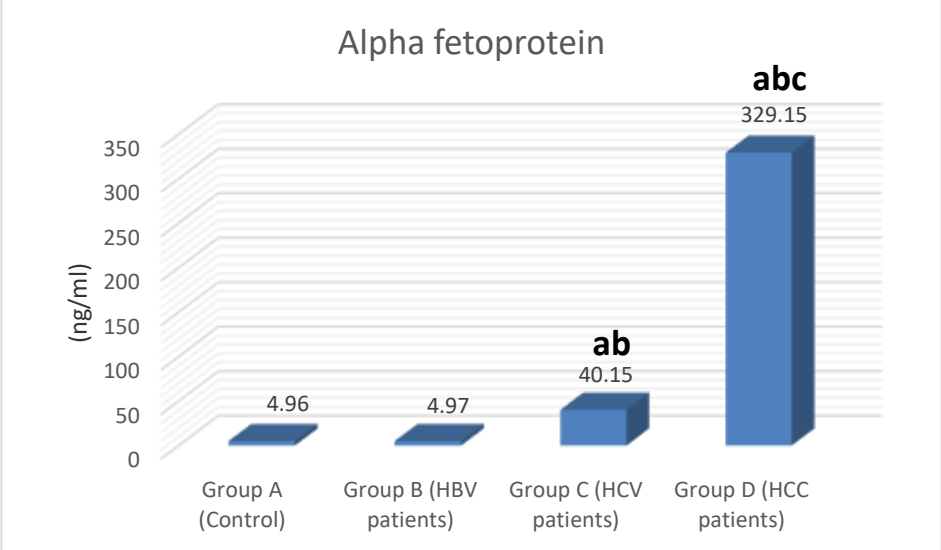
3. **α-Fetoprotein (AFP):**

Table (2) and figure (1) showed: There was no significant difference in AFP levels in HBV group as compared to the normal control group (p values 1.00), while there was significant increase in AFP levels in HCV group and HCC group as compared to the normal control (p value <0.001). There was significant increase in AFP levels in HCV group and HCC group as compared to HBV group (p value <0.001). There was significant increase in AFP levels in HCC group as compared to HCV group (p value <0.001).

Table (2): AFP (mean ± SD years) among studied groups.

	Normal Group (A)	HBV Group (B)	HCV Group (C)	HCC Group (D)
AFP	**4.96±1.82**	**4.97±1.82**	**40.15±25.69 ab**	**329.15±136.62 abc**

Figure (1): AFP (mean ± SD years) among studied groups.



4. SIRT-1:

Table (2) and figure (2) showed: There was significant increase in SIRT-1 levels in HBV group, HCV group and HCC group as compared to the normal control (p value <0.001). There was significant increase in SIRT-1 levels in HCV group and HCC as compared to HBV group (p value <0.001). There was significant increase in SIRT-1 levels in HCC group as compared to HCV group (p value <0.001).

Table (2): SIRT-1 (mean ± SD years) among studied groups.

	Normal Group (A)	**HBV Group (B)**	**HCV Group (C)**	**HCC Group (D)**
SIRT-1	**29.45±5.79**	**50.03±20.56 a**	**82.01±27.4 ab**	**140.92±45.58 abc**

Figure (2): SIRT-1 (mean ± SD years) among studied groups.

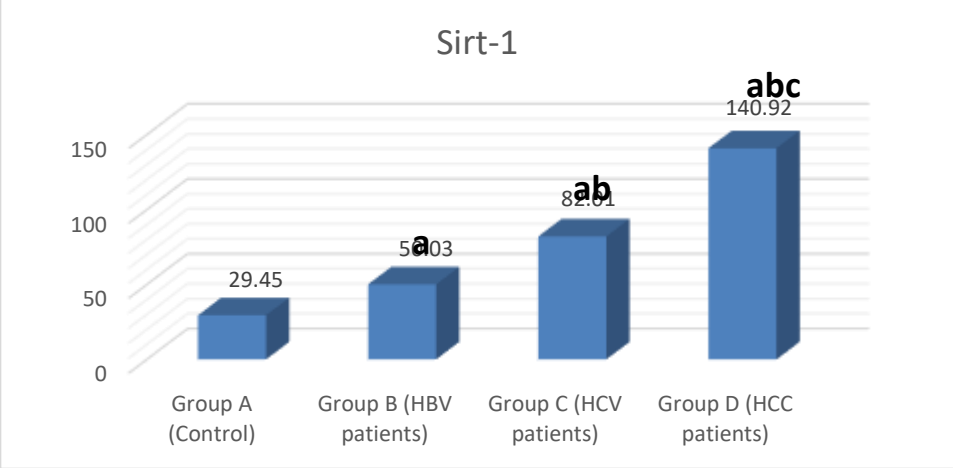


Table (3): Correlation between α- feto protein and SIRT-1 among studied groups

	α - feto protein							
	Group A		**Group B**		**Group C**		**Group D**	
	r	**p value**	**r**	**p value**	**r**	**p value**	**r**	**p value**
SIRT-1	0.175	0.279	0.028	0.865	0.077	0.636	0.355	0.024

There was positive correlation between α - feto protein and SIRT-1in group D (HCC group) only with p value 0.024. No significant correlation between α - feto protein and SIRT-1 in any other group (A, B or C) with p value 0.279, 0.865, 0.636 respectively.

Figure (3): Correlation between α- feto protein and SIRT-1 according Control group and studied groups

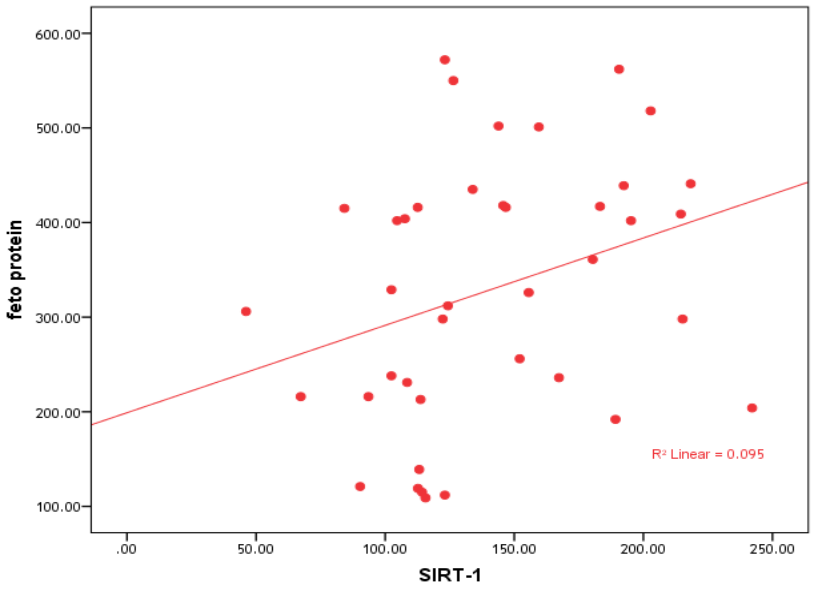
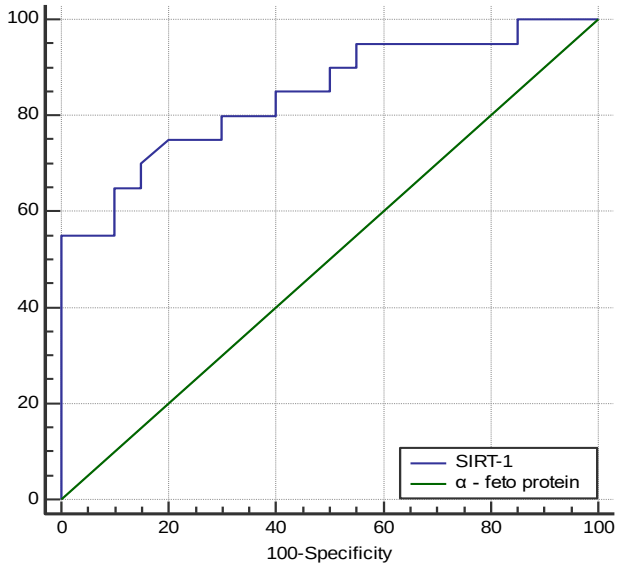


Table (4) and figure (4): Cut of point, sensitivity and specificity of α- feto protein and SIRT-1 between HBV patients group and normal group

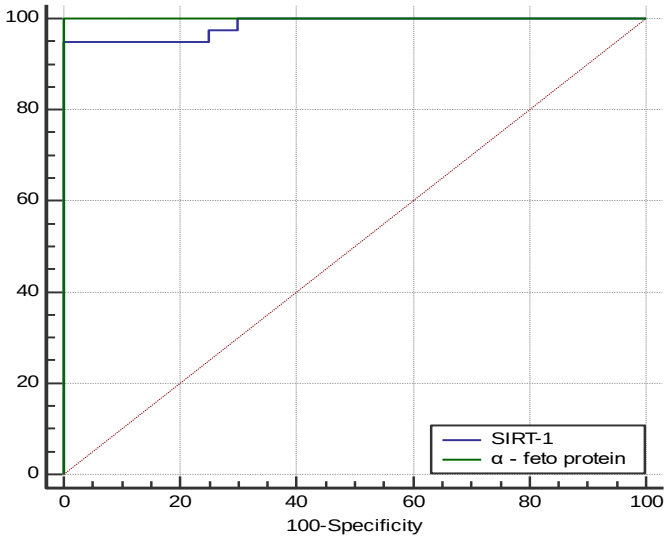
	α - feto protein	SIRT-1
Cut off point	>3.7	>34.8
AUC	0.500	0.844
Sensitivity	80.00	75.00
Specificity	20.00	80.00
+PV	50.0	78.9
-PV	50.0	76.2



- The cut of point of - feto protein and SIRT-1 = >3.7 and >34.8
- Its sensitivity is 80% and 75%
- Its specificity is 20% and 80%
- The positive predictive value is 50% and 78.9%.
- The negative predictive value is 50% and 76.2%.

Table (5) and figure (5): Cut of point, sensitivity and specificity of α- feto protein and SIRT-1 between HCV patients group and normal group

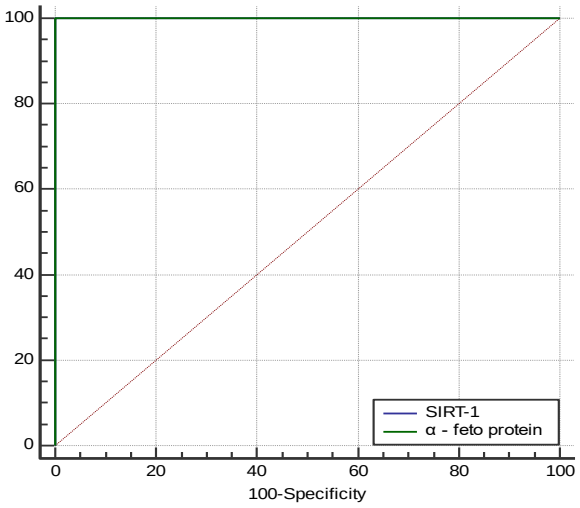
	α - feto protein	SIRT-1
Cut off point	**>10.1**	**>39.2**
AUC	**1.000**	**0.986**
Sensitivity	**100.00**	**95.00**
Specificity	**100.00**	**100.00**
+PV	**100.00**	**100.0**
-PV	**100.00**	**95.2**



- The cut of point of - feto protein and SIRT-1 = >10.1 and >39.2
- Its sensitivity is 100 % and 95 %
- Its specificity is 100% and 100 %
- The positive predictive value is 100% and 100 %
- The negative predictive value is 100% and 95.2%

Table (6) and figure (6): Cut of point, sensitivity and specificity of α- feto protein and SIRT-1 between HCC patients group and normal group

	α- feto protein	SIRT-1
Cut off point	**>10.1**	**>39.2**
AUC	**1.000**	**1.000**
Sensitivity	**100.00**	**100.00**
Specificity	**100.00**	**100.00**
+PV	**100.00**	**100.00**
-PV	**100.00**	**100.00**



- The cut of point of - feto protein and SIRT-1 = >10.1 and >39.2
- Its sensitivity is 100 % and 100 %
- Its specificity is 100% and 100 %
- The positive predictive value is 100% and 100 %
- The negative predictive value is 100% and 100 %

4. Discussion:

The objective of this study was to estimate the level of SIRT-1 in patients with HBV, HCV and HCC disease, to compare the level of SIRT-1 in the selected community, and to determine the role of SIRT-1 as a tumor marker or promoter compared to other markers, such as alpha-feto-protein.

Our study was a case-control study involving 160 people from Egypt whose age ranged from 18 to 70 years. They were recruited from the outpatient clinic for Internal Medicine at Beni-Suef University Hospital. The 160 subjects studied were split evenly into four groups; group A: stable monitoring, group B: HBV patients, group C: HCV patients and group D: HCC patients.

Our results showed that the mean age of the control group was 45.25±9.27 years, 42±9.27 years for the HBV group, 39.5±21.92 years for the HCV group, and 55±7.83 years for the HCC group. Relative with the usual control group, there was no substantial difference in age between the HBV group and the HCV group, although a significant rise in age in the HCC group compared to the control group. There was no substantial difference in the age of the HCV group in comparison to HBV group, while the age of the HCC group increased substantially compared to that of the HBV group. In contrast to the HCV community, there was a substantial rise in the age of the HCC group.

These findings were in line with [5]; who estimated that among 253 HCV patients, males accounted for 61.26 percent and females accounted for 38.74 percent, and [1] and [4]; as the former showed that 20.62 percent were females and 79.38 percent were males in HCC patients, and later showed that 67.7 percent were males and 32.3 percent were males. [6], on the other hand, found that 46.2% of patients with HBV were male and 35.2% of patients with HCV were male.

This was consistent with [6]; who showed a mean age of 55.6±10.6 years for the HBV group and 63.5±10.7 years for the HCV group. This also coincided with the findings of [1]; who showed that the mean age of HCC patients was 69.8 years, and [5]; who showed that the mean age of HCV patients was 55 years.

We recorded that 80 and 20 percent, 65 percent and 35 percent, 70 and 30 percent, and 95 and 5 percent in the control group, HBV group, HCV group, HCC group, respectively, were male and female percentages. We stated that there was no substantial gender disparity between the HBV group and the HCV group relative to the usual control group, whereas the sex difference between the HCC group and the control group was substantial. There was no substantial difference in the sex of the HCV group with respect to the HBV group, while the sex of the HCC group varied substantially

with respect to the HBV group and HCV group.

These results were in line with [5]; who estimated that among 253 HCV patients, males accounted for 61.26 percent and females accounted for 38.74 percent, and [1] and [4]; as the former showed that 20.62 percent were females and 79.38 percent were males in HCC patients, and later showed that 67.7 percent were males and 32.3 percent were males. [6]; on the other hand, found that 46.2% of patients with HBV were male and 35.2% of patients with HCV were male.

Our current study had shown that the AST and ALT levels in the HBV, HCV, and HCC groups have increased substantially relative to the standard control group. There was a substantial decrease in HCV group AST levels relative to the HBV group, while there was no significant change in HCV group ALT levels compared to the HBV group, and no significant difference in HCC and HBV group AST and ALT levels. Compared to the HCV community, there was a large rise in AST and ALT levels in the HCC group.

These results were consistent with [7]; who stated that compared to uninfected individuals, people with HBV infection had significantly higher AST and ALT. Furthermore, [6]; showed significant increases in AST and ALT levels above normal levels in patients with HBV and HCV, [1]; showed significant increases in AST and ALT levels above normal levels in patients with HCC. [5]; showed substantially increased levels of ALT and AST in patients with HCV.

Our results showed that there was no substantial difference in total and direct bilirubin compared to the normal control group in the HBV and HCV groups, while there was a substantial increase in total and direct bilirubin compared to the normal control group in the HCC group. ALP was higher than the control group in the HBV group and lower than the control group in the HCV group, with no significant difference (p values, respectively, 0.241 and 0.156). Total direct bilirubin was higher in the HCC group than in the HBV group, while, with no noticeable difference, ALP was lower in the HCC group than in the HBV group. Total, direct bilirubin and ALP levels were, without major variations, lower in the HCV group than in the HBV group. Compared with the HCV community, there were substantial increases in total and direct bilirubin and ALP in the HCC group. These were in line with [7], [8] and [9]; They showed that ALP was substantially higher than average in the HBV, HCV and HCC classes, respectively.

The mean albumin levels in the control group, HBV group, HCV group, and HCC group were shown to be 4.56 ± 0.65, 4.2 ± 0.93, 4.11 ± 2.11 and 3.36 ± 0.67 g / dl. There was no substantial change in albumin levels compared to the normal control group in the HBV group,

while there was a significant decrease in albumin levels in comparison to control group in the HCV and HCC groups. In the HCV group and the HCC group, there was no substantial difference in albumin levels relative to the HBV group. In the HCC group, there was a substantial reduction in albumin levels relative to the HCV group. This was agreed with [10]; who reported a mean albumin level of 4.55, 4.51, and 4.01 g / dl, respectively in the control group, HBV and HCC group, and [8]; who reported that the albumin level in the HCV group was slightly lower than in the control group. In addition, these findings were comparable to those of [1] and [5].

Our results showed that there was no substantial change in creatinine levels in the HBV group compared to the normal control group, while there was a significant increase in creatinine levels compared to the normal control group in the HCV and HCC groups. Compared with the HBV group, there was no substantial difference in creatinine levels in the HCV and HCC groups. Important changes in creatinine levels in the HCC group relative to the HCV group were observed. These is in line with [1] and [5] who respectively experimented on HCC patients and HCV patients and found higher than average levels of creatinine.

In our work, 5 % of patients in the HCC community were HBsAg-positive and 95 % of patients were HBsAg-negative. 95% of the patients in the HCC community were HCV-RNA and HCV AB positive, and 5% of the patients were HCV-RNA and HCV Ab negative. These findings were similar to [11]; who recorded that 12.5 percent of HCC individuals had HBV infection and 42.5 percent had HCV infection, to [1]; who showed that 18.6 percent were HBV positive among the HCC population and 38.1 percent were HCV positive. [4]; found, in comparison to our study, that 32.2% of the HCC community were HBV positive, 13.4% were HCV positive, and 54.4% were both HBV and HCV positive.

SIRT-1 levels in the HBV group, HCV group and HCC group have been shown to increase significantly relative to standard control (p value < 0.001). In the HCV and HCC groups, there was a substantial increase in SIRT-1 levels relative to the HBV group (p value < 0.001). Compared with the HCV community, there was a substantial increase in SIRT-1 levels in the HCC group (p value < 0.001). These findings were in line with [12]; who found that SIRT1 was significantly higher in HCC tissues than in adjacent normal tissues. Furthermore, [13]; reported that the relative levels of SIRT1 mRNA were significantly higher in HCC tumours than in normal control and [14]; compared to normal liver, human SIRT1 expression was elevated in HCC. In addition, these findings were comparable to

[15]; who showed that human expression of SIRT1 was elevated in HCC compared to normal liver and [16] and [17]; who noted high human expression of SIRT1.

Our findings are in line with [18] show that there is overexpression of SIRT1 in HCC. Inhibition of SIRT1 activity results in disturbance of tumor cell growth and increased expression of differentiation markers in in vitro and in vivo models. These observations suggest that SIRT1 expression promotes the growth of HCC and new treatment strategies inhibiting its activity may be a novel means for the treatment of HCC.

Also [18] said that suppression of SIRT1 in hepatocellular carcinoma cells disturbed their generation in vitro and tumor promotion in vivo. So, expression of SIRT1 increase tumorigenesis in hepatocellular carcinoma and give support to future studies aiming to suppress its action to be a new approach in treatment of HCC especially aggressive forms.

Moreover, [19] concluded that SIRT1 can have regulatory activities in liver cells to maintain energy metabolic hemostasis; it can even bring out against tumor cell initiation through the de-acetylation and activation of tumor suppressor genes. But, it may increase tumour progression if the tumor cell was already initiated, by manipulating several oncogenic transcriptional factors. Therefore, inhibiting overexpression of SIRT1 may have a role to inhibit HCC promotion. Hope so, addressing molecular mechanism of SIRT1 suppression and its effect on HCC treatment, to be approach in treatment of poorly prognosis cases of HCC.

Our results are go hand to hand with **[20]** who demonstrated that in HCC, SIRT1 was the only one of the sirtuins ever overexpressed and considered important for all stages of HCC tumor progression. And, it was frequently proofed that SIRT1 was overexpressed in tissue biopsies of HCC patient's biopsies when compared to corresponding adjacent noncancerous liver tissue and its expression was necessary for tumour progression.

On the other hand, study of [21] offer proof that SIRT1 is an essential regulator of the immune reaction that counteracts malignant HCC cell migration as well as growth, indicating that macrophage SIRT1 could serve as an innovative target to treat HCC.

5. Conclusion and Recommendations:

Our study demonstrated that SIRT-1 is overexpressed in HCC patients compared to chronic noncancerous viral liver parenchyma diseases, so it may be included in pathogenesis of HCC. SIRT-1 may have matchable specificity and sensitivity as AFP in HCC patients.

From therapeutic view inhibiting the expression and activity of SIRT1 might have a therapeutic effect to handle HCC.

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