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***Correlation between SREBP-1c and Prominent Nucleoli Hepatocytes of Type 2 Diabetic Rat Model Induced by Dietylnitrosamine***

Korelasi antara SREBP1c dan Hepatosit Bernukleoli Prominen pada Tikus Diabetes Tipe 2 yang Diinduksi dengan Dietylnitrosamine

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**Abstract**


Type 2 Diabetes Mellitus (T2DM) demonstrates relative risk of cirrhosis towards liver cancer. Although the relationship between type 2 diabetic and cirrhotic toward HCC has been suggested, the association between SREBP-1c and the number of prominent nucleoli hepatocytes has not clearly explored. This study aims to prove the correlation between those variables by using male Wistar rat model given fat diet continued with administration of Streptozotocin injections twice in low dosage and an injection of dietylnitrosamine once a week for 10 weeks. At week 16, they were sacrificed, the fresh liver tissue was processed for examining the expression of SREBP-1c, while the fixative paraffin block was sliced and stained with Hematoxylin Eosin to count the number of prominent nucleoli hepatocytes. The western blotting result demonstrated increasing level of SREBP-1c in diabetic rat significantly. The liver sections showed nodules consisting of pleomorphic cells, inflammatory cells, and prominent nucleoli hepatocytes. The correlation between level of SREBP-1c and total number of prominent nucleoli hepatocytes was measured with non-parametric correlation method statistically. It demonstrated coefficient  $r=0,435$  with  $p$  value= $0,037$ . It is concluded that increasing number of prominent nucleolus hepatocytes related to enhancing level of SREBP-1c in the process of T2DM towards Hepatocellular Carcinoma.

**Keywords:** type 2 DM; diethylnitrosamine; HCC; streptozotocin; Wistar rats

**How to Cite:**

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### Abstrak

DM tipe 2 (DMT2) menunjukkan risiko terjadinya sirosis menuju kanker hati. Walaupun hubungan antara diabetes tipe 2 dan sirosis hati menuju kanker hati telah dilaporkan, namun korelasi antara ekspresi SREBP-1c dengan jumlah hepatosit bernukleoli prominen belum diungkapkan dengan jelas. Riset ini mempunyai tujuan untuk mengetahui korelasi antara kedua variable diatas mamakai hewan model tikus jenis Wistar yang diberi makanan lemak, diinjeksi dengan streptozotocin dosis rendah sebanyak 2 kali, dilanjutkan dengan injeksi diethylnitrosamine sekali dalam seminggu selama 10 minggu. Pada minggu ke-16, semua tikus dikorbkan, organ hati dikeluarkan, sebagian jaringan hati segar diproses untuk pemeriksaan SREBP-1c, sementara jaringan hati yang telah diblok dipotong dan diwarnai dengan pewarnaan hematoxylin-eosin, untuk menghitung jumlah hepatosit dengan nucleoli yang prominen. Hasil pemeriksaan menunjukkan peningkatan kadar SREBP-1c yang bermakna pada kelompok tikus diabetes. Pada sediaan hati tampak nodul-nodul yang terdiri dari sel nekrotik, sel radang dan hepatosit dengan nukleoli yang prominen. Korelasi antara kadar SREBP-1c dan jumlah hepatosit dengan nukleoli yang prominen yang dianalisis secara statistik menggunakan rumus korelasi non-parametrik menunjukkan keeratan korelasi  $r=0,435$  dan kebermaknaan dengan nilai  $p=0,037$ . Disimpulkan bahwa peningkatan jumlah hepatosit bernukleoli prominen dikarenakan peningkatan kadar SREBP-1c pada proses DMT2 dapat berlanjut menjadi HCC.

**Kata kunci:** DM tipe 2, diethylnitrosamine, HCC, streptozotocin, Wistar rats

### Introduction

Global Burden Disease reported that from 1990 to 2019, the incidence and mortality of type 2 diabetes increased by 49% and 10.8%, respectively. The incidence of T2DM is higher in men and increasing in age from 75 to 79 years.<sup>1</sup> The International Diabetes Federation estimates that 463 million people worldwide over the age of 20 and older are suffered from diabetes and then will be increased to 700.2 million by 2045.<sup>2,3</sup> Diabetes alone kills more than one million people each year, making it the ninth leading cause of death. Gender distribution is balanced, peaking around age 55.<sup>4</sup> Worldwide, the prevalence of T2DM and various cancer kinds is rising quickly. Type 2 Diabetes Mellitus was associated with increased risk for liver cancer especially Hepatocellular Carcinoma in United State either in men or women. Several investigations showed that there is still a strong link between T2DM and a risk of HCC.<sup>5</sup> In Asian countries, increases the proinflammatory cytokines NF-kB, TNF-a, IL-6, and IL-1b, which are linked to cell death and oxidative stress suggest that T2DM may induce hepatic inflammation that progressing HCC.<sup>6</sup> Hepatocellular Carcinoma is accelerated by co-morbidities such obesity and type 2 diabetes, as well as nonalcoholic fatty liver disease (NAFLD). Incidence of HCC linked to metabolic disorders has grown during the past ten years; it makes that molecular mechanisms connect T2DM and HCC with pathologic characteristics need to be explored. Pathogenic variables linked to the

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etiology of HCC included abnormal metabolites, inflammatory agents, and immunological modulations.<sup>7</sup>

Liver cancer is known as HCC, a malignancy tumor, affects about 90% of patients, and T2DM is believed to be an emerging risk factor. The second greatest cause of cancer-related deaths globally is hepatocellular carcinoma (HCC), a primary liver cancer that is the seventh most prevalent cancer in the United State, is responsible for leading cause of deaths. The incidence has been rising worldwide over the last 20 years and is expected to increase until 2030. The prognosis is poor due to its rapid infiltrating growth and cells transformation.<sup>8</sup> Chronic hepatitis B and C, alcoholism, metabolic liver disease, diabetes, and nonalcoholic fatty liver disease are all risk factors for HCC. All of these risk factors have the potential to be avoided, demonstrating the great potential of risk reduction for reducing the prevalence of HCC worldwide.<sup>9</sup>

In T2DM and NAFLD, releasing numerous pro-inflammatory and vasoactive factors influenced HCC development.<sup>10</sup> Pro-inflammatory cytokines may contribute to the development of HCC through the chronic inflammation associated with DM. The hepatic stellate cells are stimulated to secrete matrix proteins in liver fibrosis due to hyperinsulinemia in T2DM. Risk factors for HCC such as NAFLD, NASH, obese, and T2DM have been reported to contribute to the development of HCC and count for about 37% in the US. As a result, the prevalence of metabolic risk factors, including metabolic syndrome, obesity, and type II diabetes, has become a major cause of HCC.<sup>11,12</sup> Elevated glucose and saturated fatty acid levels in T2DM stimulate AMP-activated protein kinase, and the consequences contribute to increase the expression of SREBP1c.

In the liver, SREBP-1c and ChREBP, the major transcriptional regulators, promote adipogenesis, which is thought to be a key cause of HCC. SREBP-1c is a family of transcription factors that regulate lipid homeostasis from glucose in the liver. It is also a mediator of insulin effects and its function in the control of hepatic lipogenic and glycolytic genes. It was reported that inhibiting the SREBP-1c pathway dramatically controls metabolic strategy in HCC in diethyl nitrosamine-induced HCC.<sup>13,14</sup> Recent data suggest that SREBP-1c activation stimulates adipogenesis in fatty liver by insulin at both transcriptional and post-translational levels through complex signaling cascades.<sup>15</sup> SREBP-1c can regulate the biosynthesis of fatty acid, triglyceride and cholesterol then is able to cause fatty liver.<sup>16</sup> Thereby to know the role of various factors in SREBP1 pathway is very important. A well-known nuclear transcription factor called SREBP-1c plays roles in lipid synthesis, tumor cell proliferation, and apoptosis. Recent research demonstrated a link between nonalcoholic fatty liver disease and an increased risk of HCC, particularly nonalcoholic steatohepatitis. These results imply that aberrant lipid metabolism may

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be crucial to the development of HCC.<sup>17</sup> Epidemiological studies suggested that NAFLD, which typically develops in type 2 diabetes, has been reported as the greatest proportion of risk factors for HCC. Thereby it is considered the most prevalent type of chronic liver disease, which is increasing among Americans with type 2 diabetes in general.<sup>18</sup> Changes in the hepatic microenvironment have a significant impact on HCC development and progression, with cytokines playing a crucial role in these changes. Given the worldwide significant rise in type 2 diabetes and liver cancer incidence, studying the pathophysiological associations between T2DM and risk of cancer, remains as great clinical importance, however the pathogenic mechanisms linking these diseases to liver cancer are still poorly understood. The advancement of liver cancer is mostly mediated by the inflammasome-induced cytokines linked to both disorders.<sup>19</sup> T2DM and HCC have been linked to obesity, decreased insulin sensitivity, and NAFLD, which provides evidence for a strong connection between the two conditions. Fibrosis is not necessary for the occurrence of HCC in NASH patients, suggesting that insulin resistance and fat may operate as direct mediators of carcinogenesis. Increased levels of interleukin-6, leptin, and tumor necrosis factor (TNF) are caused by insulin resistance and obesity, which worsens the condition. These high adipocytokine levels also increase angiogenesis, cell proliferation, and apoptosis inhibition.<sup>20</sup> Strong evidence that type 2 diabetics are more likely to develop HCC with severe histology has emerged during the past ten years.<sup>21</sup>

Liver parenchyma consists of parenchyma, sinusoidal cells and perisinusoidal astrocytes. Most liver cells are hepatocytes (60-80%) are epithelial cells with a diameter average 25µm. In portal tract, there are two vascular, hepatic arteries and veins and hepatic biliary. Kupfer cells as macrophages make up 10% of liver cells, protecting foreign substances by phagocytosis. Pit cells make up 2-5%, belong to granular lymphocytes and have secretory granules. Ito cells (fat-accumulating cells) make up 3%, located in perisinusoidal space. Interrelationships between hepatocytes in inflammatory processes leading to auto/paracrine regulation that activates the processes that result in fibrosis.<sup>22</sup>

Diethyl nitrosamine (DEN)-induced mouse was reported to create HCC by injected i.p. with dosage 75 mg/kg body weight and 100 mg/kg body weight. HCC was developed throughout the 32-week experimental period. Parenchymal cells changed in size and shape and had hydrophic and fatty degeneration. Pseudo-nodules developed, indicating a later stage of liver carcinogenesis, with a progressive rise in the number of transformed hepatocytes, characterized by large round nuclei and prominent nucleoli, pleomorphic nuclear per cytoplasmic ratio (N/C ratio) enlargement, and hyperchromatic nuclei.<sup>23</sup> Type 2 diabetes in animals is modeled to closely resemble the disease. Streptozotocin was administered twice weekly to Wistar rats that had been

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receiving HFD for four weeks. This medication causes insulin resistance and significantly lowers -cell activation.<sup>24</sup>

Although SREBP-1, as nuclear transcription involved in lipogenesis of T2DM reported an important part in the development of HCC, however relationship between level of SREBP-1c and the number of prominent nucleoli hepatocytes has not yet been clear explored to date. Consequently, this study's goal is to explore the correlation between SREBP-1c and prominent nucleoli hepatocytes using animal model of T2DM induced with DEN.

### Methods

In the animal facility of the Mochtar Riady Institute for Nanotechnology, all animal experimentation protocols were carried out in accordance with guidelines for the care and use of laboratory animals from the National Institutes of Health. This research protocol was released from Ethics Committee of the Mochtar Riady Institute of Nanotechnology, Indonesia, under protocol number 01.1803185.

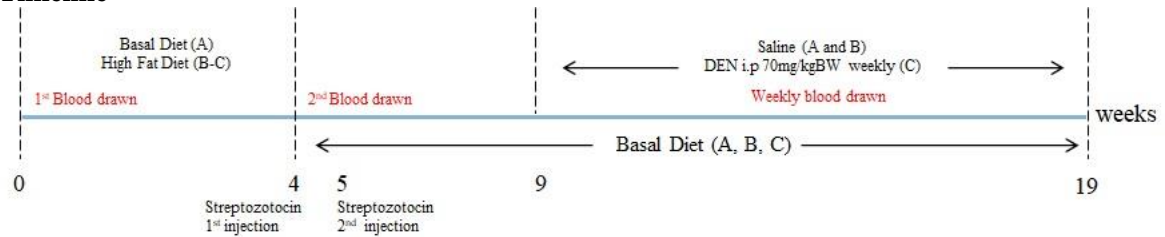
All rats were kept in cages made by polycarbonate plastic (one cage for 2 rats) with shaved bedding at room temperature of  $25 \pm 2$  °C,  $50 \pm 10\%$  humidity with 12 hours of a cycle light-dark. Using male Wistar rats due to they are appropriate as a diabetes animal model, and their island of  $\beta$  cells are more prone to streptozotocin-induced cytotoxicity.<sup>25,26</sup> After adjusting for the success rate of developing diabetes, the number of samples in each group was calculated using Federer's formula with an 80% rate of success in causing diabetes. The diabetic induction of rats was performed according to our previous study.<sup>24</sup> After acclimatization for a week, all 18 rats were randomly divided into 2 groups. First control group (n=6) was fed with a basal diet purchased from Institute of Food Technology, Bogor Agricultural University, Bogor, Indonesia. They continuously injected with sterile saline during the experiment. In the second group (n=12), high fat diet (HFD) were administered for the first 4 weeks and continued with a basal diet. In the fifth week, the rats in second group were injected with streptozotocin (STZ) 45mg/kg body weight (BW) intra-peritoneally (IP) twice at a one-week interval. In week 5 after injection, these second group were divided into 2 subgroups, i.e., diabetic group A (n=6) which continue injected with saline IP for 10 weeks; while diabetic group B (n=6) injected IP with DEN 70 mg/kg body weight for 10 weeks given weekly to induce hepatocellular carcinoma.<sup>23</sup>

At weeks 0, 4, 5, and 9, fasting blood glucose was measured by Glucose test strip drawn from the tail after an overnight fast of 10 hours. (Easy Touch, manufactured by Biopic Technology, Inc., number 188, Jhonghua south road, Gongguan Village, Jhunan Township, Miaoli Country, Taiwan). At week 20, all rats were given a combination of xylazine and ketamine

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at doses of 40–80 mg/kg body weight and 5–10 mg/kg body weight, respectively, to put them to sleep. They were then all exsanguinated to end their lives. The liver was entirely removed, weighed, and split into two portions: one portion was used for fresh liver tissue for protein analysis, while the other portion was fixed in neutral buffered formaldehyde for histological analysis.

### Timeline



### Analytical for Histology

After being fixed in buffered neutral formalin 10%, the livers were dehydrated in a succession of alcohol concentrations ranging from 70% to 100%, submerged in xylene, and finally embedded in paraffin. The paraffin block was sliced at a thickness of 4  $\mu$ m, deparaffinized in xylene, and rehydrated using ethanol concentrations ranging from 100% to 70%. Hematoxylin Eosin used to stain the sections and for observing the general structure of liver lobules and identified the prominent nucleoli hepatocytes.

To count the number of prominent nucleoli hepatocytes using Fiji app, 40 high-magnification microscope fields were examined, and each section contained 10 randomly selected, non-overlapping fields. The number of prominent nucleoli hepatocytes were analyzed as a percentage of 500 hepatocytes.<sup>28</sup> Collagen deposition and septum formation were stained with Masson's Trichrome. Sections were mordanted with Bouin's solution and washed with tap water until clear. The slides were then washed under running water after being stained with Weigert's hematoxylin. Aniline blue was used to stain sections before being rinsed with distillate water and Biebrich's scarlet fuchsin solution. Lastly, slides was differentiate using 1% glacial acetic acid and processed to be mounted.<sup>29</sup> Assessment of histopathology changes were confirmed by two independent pathologists to determine the features of prominent nucleoli hepatocytes and fibrotic septa.

### Western Blotting

SREBP-1c level were extracted and the concentration-adjusted liver proteins were separated by SDS-PAGE using a 10% gel and transferred to 0.45  $\mu$ m nitrocellulose blot absorbent

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filter paper sandwiches (Biorad, California, U.S.A). Membranes were washed with phosphate buffered saline - 0.1% Tween 4 x 5 minutes each, then block with 5% skim milk for 1 hour at room temperature. The membrane then incubated with Rabbit polyclonal antibody to SREBP1 1:500 (Abcam, Cambridge, U.K) and Rabbit polyclonal to beta Actin 1: 1000 (Abcam, Cambridge, U.K) overnight at 4°C, washed 4 x 5 minutes with PBS-T and incubated with anti-rabbit 1:10.000 (Abcam, Cambridge, U.K) for 1 hour at room temperature. After 4 x 5 min washes with PBS-T, membranes were stained with Western Bright ECL HRP substrate (Advansta, San Jose, U.S.A) then captured with VersaDoc (Biorad, California, U.S.A) after 180 seconds exposure. The protein level was measured semi quantitatively using ImageJ software.<sup>30</sup>

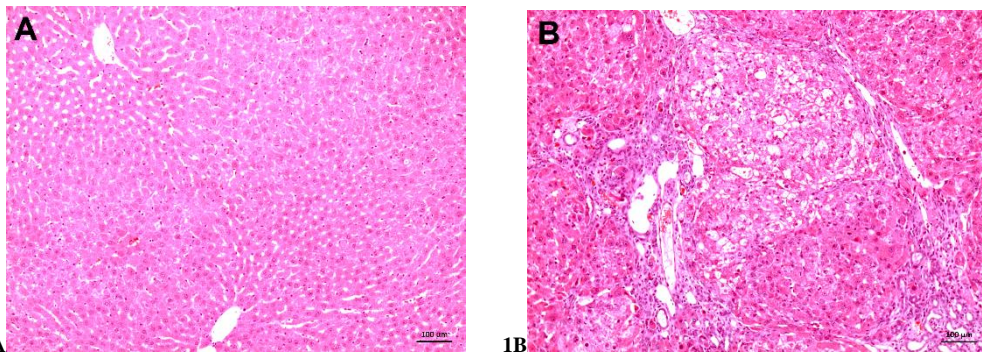
### Analytical Statistic

The mean and standard deviation of the mean are used to express all data. Based on the Saphiro-Wilk normality test, the statistical analysis of SREBP1c and the numbers of notable hepatocytes was examined for normality. The number of hepatocytes with conspicuous nucleoli and mean differences in SREBP-1c levels were examined using Mann-Whitney utilizing SPSS software version 23 (IBM, Armonk, NY, USA). Spearman's rho correlation was used to assess the relationships between the variables. As statistical significance, a p-value of 0.05 or less was considered acceptable.

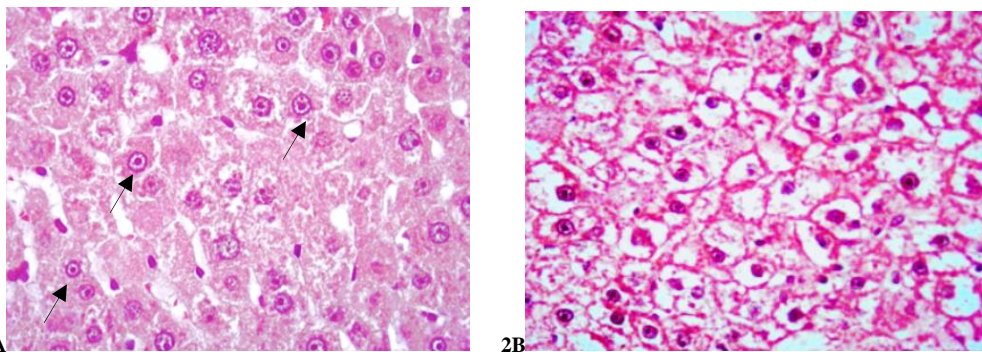
### Results

Hematoxylin Eosin staining for control liver sections (group A) showed one-cell-thick classical hexagonal liver lobules radiating from the lobules' perimeter to the hepatic central vein. Liver sinusoids were seen between adjacent plates. The liver section of diabetic rats (group B) showed a normal general architecture with dilated sinusoids and vacuolation in some of hepatocytes were shown in figure 1A. Liver sections of diabetic-den rats (group C) revealed disruption of liver architecture with necrotic hepatocytes, increased infiltration of lymphocytes surrounded by condensed eosinophilic cytoplasm. Liver nodules consisting pleomorphic cells, enlargement of hepatocytes with high nucleus to cytoplasmic ratio, nucleus darkening and prominent nucleoli hepatocytes with chromatin condensation were observed and shown in figure 1B. The section of diabetic rats in group B shown abundance of prominent nucleoli hepatocytes (Fig. 2A) while liver sections of diabetic-den rats in group C demonstrated pleiomorphic and necrotic hepatocytes within the nodule (Fig 2B).

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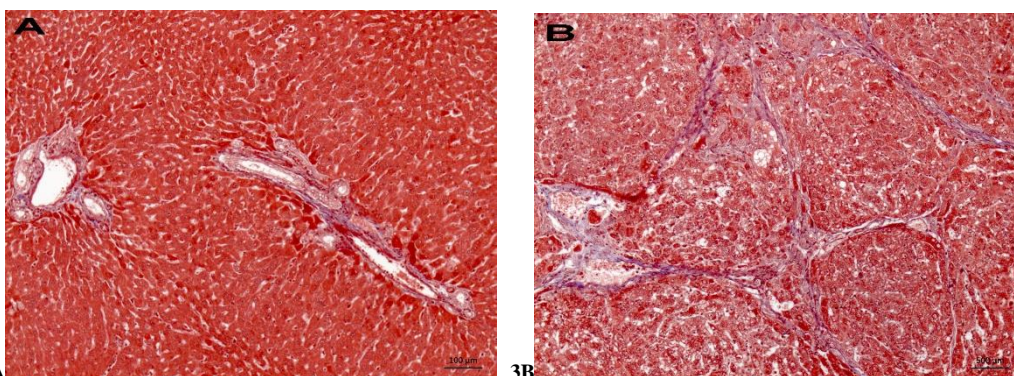


**Figure 1A.** the liver section of diabetic rats showed arrangement structure of normal hepatocytes radiate as one line towards central vein. **1B.** section of diabetic-den rats showed disarrangement of hepatic plate, unclear lobular architecture, dilated sinusoids, multiple nodules, enlargement of hepatocytes with hyperchromatic nuclei and prominent nucleoli of hepatocytes were found within the nodules. Magnification 100x objectives lens.



**Fig 2A** Activated hepatocytes scattered in liver parenchyma of diabetic rats characterized with prominent nucleoli (arrow). **2B** pleiomorphic and necrotic hepatocytes with clumping of chromatin and prominent nucleoli were found within the nodule in diabetic-den rats. 400x magnification

The liver sections of control normal rats stained with Masson's trichrome showed thin borders of collagen around central veins, portal tracts, and sinusoids. Liver sections of the diabetic rats in group B had slightly increased deposition of collagen fibers around the central and portal veins (Fig.3A). Liver sections of diabetic-den group C had markedly increased collagen deposition, which accumulated peri-vascular and within the portal vein. Thick collagen septa were seen bridging portal to portal/central veins to form pseudo nodules (Fig.3B).



**Fig 3A.** Masson Trichrome staining of liver of diabetic rats showed the collagen fibers surrounding central and portal veins. **3B.** Liver section diabetic-den group of rats showed thick connective tissue fibers surrounded portal and central veins and septa bridging over to form pseudo nodules. Magnification 100x objectives lens.



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**Expression of SREBP 1c**

The protein level of SREBP-1c was measured semi quantitatively using ImageJ software. Semiquantitative expression of SREBP-1c among the groups of control (A), diabetic (B) and diabetic den (C) analyzed using one-way ANOVA showed significantly difference with  $p < 0.05$  (Table 1). SREBP-1c protein's average concentration in group A was  $0.06 \pm 0.02$ , while in group B and C were  $0.41 \pm 0.08$  and  $0.23 \pm 0.11$  respectively. The comparison within groups analyzed with Bonferroni T-test significantly showed differences (Table 2).

The number of prominent nucleoli hepatocytes were assessed using Fiji app in 10 random fields non-overlapping each section. Prominent hepatocytes characterized with dense nucleoli were calculated as a percentage of 500 hepatocytes.<sup>26</sup> Assessment were confirmed by two independent pathologists to determine the features of prominent hepatocytes. The differences number of prominent nucleoli hepatocytes among the groups analyzed using One way ANOVA showed significantly difference with  $p < 0.01$  (Table 3). The mean level of prominent nucleoli hepatocytes in control group was  $0.14 \pm 0.03$ , while in group B and C showed increasing with number  $0.39 \pm 0.08$  and  $0.55 \pm 0.07$  respectively. The comparison within control group to groups B and C done with T-test significantly showed differences (Table 4).

**Table 1 The Expression of SREBP-1c Analyses with ANOVA**

No	Group	Mean±SD (Relative intensity)	Range	p-value
1	A	$0.06 \pm 0.02$	0.04-0.08	<0.05
2	B	$0.41 \pm 0.08$	0.33-0.49	
3	C	$0.23 \pm 0.11$	0.12-0.34	

**Table 2 Expression of SREBP-1c Analyses with Bonferroni T test**

No	Group	Mean±SD (Relative intensity)	Range	p-value
1	A	$0.06 \pm 0.02$	0.04-0.08	0.001 (to Group B) 0.009 (to Group C)
2	B	$0.41 \pm 0.08$	0.33-0.49	0.022 (to Group C)
3	C	$0.23 \pm 0.11$	0.12-0.34	0.022 to (Group B)

**Table 3 The Number of Prominent Nucleoli Hepatocytes Analysis with ANOVA**

No	Group	Mean±SD (Relative intensity)	Range	p-value
1	A	$0.14 \pm 0.03$	0.11-0.17	<0.01
2	B	$0.39 \pm 0.08$	0.31-0.47	
3	C	$0.55 \pm 0.07$	0.48-0.62	

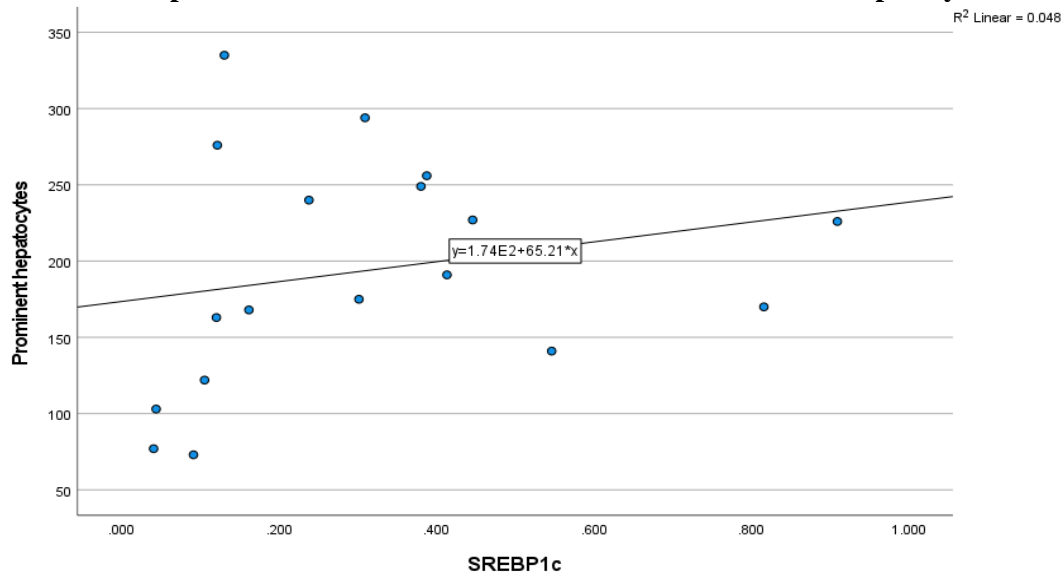
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The data of SREBP1-c and numbers of prominent nucleoli hepatocytes were analyzed using the Saphiro-Wilk normality test, the data were examined for normality. With SPSS software version 23 (IBM, Armonk, NY, USA), mean differences between SREBP-1c level and the overall number of hepatocytes with conspicuous nucleoli were compared using Mann-Whitney analysis. Scatter plot of the SREBP-1c and prominent nucleoli hepatocytes showed the relationship between SREBP-1c and Prominent Nucleoli Hepatocytes in a group of subjects. The plot reveals a positive correlation between the two variables, as the value of SREBP-1c increases, the value of Prominent nucleoli Hepatocytes tends to increase as well (graph 1). Spearman's rho correlation was used to assess the relationships between the variables, and the results showed p-values = 0.037 and coefficient correlation  $r = 0.435$ . (Table 5). This correlation is statistically significant, indicating that it is unlikely to have occurred by chance.

**Table 4 The Number of Prominent Nucleoli Hepatocytes Analysis with T-test**

No	Group	Mean±SD (Relative intensity)	Range	p-value
1	A	0.14±0.03	0.11-0.17	0.002 vs Group B 0.000 vs Group C
2	B	0.39±0.08	0.31-0.47	0.016 vs Group C
3	C	0.55±0.07	0.48-0.62	0.016 vs Group B

**Graph 1 Scatter Plot SREBP-1c with Prominent Nucleoli Hepatocytes**



**Table 5 The Correlation between SREBP-1c and Prominent Nucleoli Hepatocytes**

Variable 1	Variable 2	Coefficient r	p-value
SREBP1c	Prominent hepatocytes	0.435	0.037

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### Discussion

In this study, the protein level of SREBP-1c in group B showed the highest expression compared to the other groups with p value=0.00. In T2DM, elevated glucose and saturated fatty acid levels stimulate AMP-activated protein kinase, and the consequences contribute to increase the expression of SREBP-1c, which promotes adipogenesis. A particular transcription factor, SREBP-1c controls the insulin- and glucose-dependent process of adipogenesis, and stimulates fatty liver. Reduces SREBP-1c activation, reduces adipogenesis, and improves fatty liver and insulin sensitivity.<sup>15,16</sup>

Liver specimens of diabetic rats' groups stained with Hematoxylin and Eosin (HE) demonstrated classical hexagonal liver lobules with radiated from hepatic central vein in the center of lobule toward periphery of the lobules with the presence of vacuolation in some of hepatocytes that was similar with the normal architecture of the liver. These similar features have been reported in mouse and rat streptozotocin-induced diabetes models.<sup>31</sup> On the contrary, the liver sections of diabetic-den group C showed multiple nodules, necrotic hepatocytes, pleomorphic cells, activated hepatocytes characterized with enlargement of hepatocytes, hyperchromatic nuclei, and prominent hepatocytes. DEN-induced mice were reported to create liver carcinogenesis leading to hepatocellular carcinoma in a period of 20 and 32 weeks.<sup>23</sup> Strong evidence that type 2 diabetics are more prone to develop HCC with more severe histologic types has emerged during the past ten years.<sup>21</sup> Liver section of diabetic-den rats' figures is similar with microscopic description of well differentiated HCC characterized with dilated sinusoids, lymphocytes infiltration, multiple nodules. It exhibits a higher N/C ratio than normal, round nuclei with rough chromatin and prominent nucleoli.<sup>32</sup> According to Yousef et al., DEN induction led to many alterations in hepatocytes, including necrosis with inflammatory cells. This result is consistent with their findings.

In this study, correlation between SREBP-1c and prominent nucleoli hepatocytes showed moderate coefficient correlation with  $r=0.435$  and p value= 0.037. The correlation between SREBP1c and prominent nucleoli hepatocytes supports that type 2 diabetes contribute to similar features of HCC development. It was reported that increased prevalence of NAFLD, NASH, obesity, and type 2 diabetes stimulate to the growth of HCC.<sup>9</sup> Insulin resistance causes insulin-like growth factor 1 (IGF1) levels to rise, which in turn activates the PI3K and MAPK signaling pathways, promotes cell development, and inhibits apoptosis.<sup>33</sup>

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### Conclusion

In our study, the expression of SREBP-1c and number of prominent nucleoli hepatocytes increased significantly and demonstrated moderate coefficient correlation. It is suggested that T2DM induced expression of SREBP-1c as lipogenesis factor that stimulates the transformation of prominent nucleoli hepatocytes in the development of HCC. It is concluded that SREBP-1c, as lipogenesis transcription factor, enhancing their expression in T2DM may be crucial in the development of HCC by increasing the proportion of hepatocytes with prominent nucleoli.

In the future study, it is important to evaluate the association between administration of metformin to patients with NAFLD, since metformin as inhibitor of insulin resistance inhibits the progression of fibrosis in NAFLD toward HCC.

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