Study of Cellular Senescence and Vitamin D Deficiency in Nonalcoholic Fatty Liver Disease and The Potential Protective Effect of Vitamin D Supplementation

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Background: **Nonalcoholic fatty liver disease (NAFLD) is a pathological process characterized by excessive hepatic fatty deposition with possible involvement of vitamin D deficiency and cellular senescence. The aim of this study is to investigate the pathophysiologic role of vitamin D deficiency and cellular senescence in NAFLD development. Moreover, it aims to investigate the potential protective role of vitamin D supplementation.** ***Methods:*** **This is an experimental Case/Control study. Forty-five male albino rats were enrolled in this study. Animals were divided into four groups: negative and positive control groups (10 for each group), a model of NAFLD (11) and vitamin D–treated NAFLD groups (14). At the end of the experiment, all rats were subjected to the following investigation; biochemical estimation of serum 25 hydroxycholecalciferol, senescence marker protein–30 (SMP-30), lipid profile and calculation of homeostatic model of insulin resistance (HOMA-IR).** ***Results:*** **NAFLD group shows a significant increase in glucose, insulin levels, and HOMA- IR compared with both normal controls. This finding indicates the intimate association between insulin resistance and NAFLD pathogenesis. Moreover, it was found that NAFLD group shows a significant decrease in SMP-30 level compared with normal controls. While vitamin D–treated NAFLD group shows significant increased SMP-30 and decrease in HOMA-IR in comparison with nontreated NAFLD group.** ***Conclusion:*** **Vitamin D deficiency and increased cellular senescence are key features of NAFLD. Vitamin D supplementation could play a protective role, which needs further investigation including clinical human study. (J Clin Exp Hepatol 2021;11:219–226)**

NAFLD

Nonalcoholic fatty liver disease (NAFLD) signifies a range of pathologies commencing with simple hepatocytes' triglyceride accumulation beyond 5% of the liver weight to fibrosis, followed by cirrhosis, which eventually escalates the danger of hepatocellular carcinoma.[1] Steatohepatitis (NASH) is an advanced form of NAFLD. The pervasiveness of NAFLD varies between 6.3% and 33% in the general populace, while that of NASH ranging from 3% to 5%.[2] NAFLD remains the leading reason for chronic liver disease among people in the Kingdom of Saudi Arabia; exceeding even viral hepatitis[3] at incidence rate 12.6%.[4]

Cellular senescence has been described as an irreversible cell-cycle dysregulation merged with pro-inflammatory cytokines secretion and mitochondrial dysfunction that end in cellular aging. Cellular senescence has been said to complicate hepatic steatosis.[5]

It is proposed that vitamin D deficiency could be linked to IR-related states like metabolic syndrome and obesity.[6] So, vitamin D deficiency could be linked to NAFLD as it is considered the hepatic manifestation of metabolic syndrome. However, the association between vitamin D status and NAFLD is still debatable.[7]

The current study aims to investigate the pathophysiologic role of vitamin D deficiency and cellular senescence in NAFLD development. Moreover, it aims to investigate the protective role of vitamin D supplementation. The objectives of the current study are designed to examine the status of senescence marker protein −30 and 25 hydroxy vitamin D_3 in an experimental rat model of NAFLD. Moreover, it is designed to investigate the possible correlation between (SMP-30) and vitamin D status in NAFLD rat models. Besides that, this study is expected to investigate the possible protective role of vitamin D supplementation.

Keywords: NAFLD, vitamin D, cellular senescence, HOMA-IR, SMP-30
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Abbreviations: NAFLD: nonalcoholic fatty liver disease; HOMA-IR: homeostatic model of insulin resistance; SMP-30: senescence marker protein - 30; NASH: steatohepatitis; IR: insulin resistance; ALT: alanine transaminase enzyme; AST: aspartate transaminase enzyme; ALP: alkaline phosphatase; GGT: γ glutamyltranspeptidase
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METHODS

Ethical Considerations

This protocol was approved by the unit of Biomedical Ethics-Research committee (Reference No.10–19) Animal Study, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia. The experiment was carried out in accordance with the animal welfare act and guide for care use of Experimental Research Center, King Abdulaziz University.

Methods and Study Design

The study is an experimental animal study. This study was conducted during the period from March 2018 to December 2019 in the Department of Clinical Biochemistry, Faculty of Medicine, King Abdulaziz University, Jeddah.

Animals and Experimental Protocol

Forty-five adult male Sprague–Dawley albino rats, weighing approximately 120 ± 20 g, were obtained from the animal house, Faculty of Pharmacy, King Abdulaziz University and enrolled in this study. The animals housing condition was as follows: 24 °C ± 3 °C temperature,40–70% humidity and 12/12 h light/dark cycle. They were fed on commercial food ad-libitum (standard laboratory rat chow), and the access to drinking water was allowed freely for the first 10 days following delivery to allow acclimation to the new environment.

The rats were classified into four main groups as follows: Group I: served as a negative control group included 10 albino rats with a normal diet. They were given water ad-libitum and were fed a standard chow with 26.5% protein, 3.8% fat, 40% carbohydrate, and 4.5% crude fiber in 100 g of chow.[8] Group II: served as an NAFLD animal group (non-treated NAFLD group model) included 11 albino rats. Rats were fed a high-fat diet: 2%, cholesterol, 0.5% sodium chlorate, 0.2% propylthiouracil, 5% sucrose, 10% lard, and 82.3% as fatty acids. The induction of NAFLD was carried out in 8 weeks' duration according to the method described by Tan *et al.*[9] Group III: served as a positive control group included 10 albino rats. The animals were fed normal diet and supplemented with vitamin D at 2.5 mg/kg single dose daily, dissolved in olive oil (0.75 ml of vitamin D contain 300IU as the concentration is 400IU/ml;120 IU/mg).[10] Group IV: included 14 rats vitamin D treated rats with NAFLD. (NAFLD + Vit. D) at 2.5 mg/kg single dose daily, dissolved in olive oil (0.75 ml of vitamin D contain 300 IU as the concentration is 400IU/ml;120 IU/mg).[9,10]

Female or nonalbino rats that are weighing more than (200 g) at the start of the research experiment and rats that failed to gain expected weight were excluded from the study.

Anthropometric Measurement

Weekly measurement of height in centimeters and body weight in grams were carried out, and body mass index (BMI) was calculated according to Novelli *et al.*[8] Hepatic Index = liver weight (g)/total body weight (g) was also carried out.

Sampling and Biochemical Investigations

At the end of the experimental protocol duration, after 12 h overnight fasting, a blood sample was withdrawn from each rat using a disposable plastic syringe from retro-orbital venous plexus (under a complete aseptic condition). The samples were collected into a plastic plain container, and sera were collected after centrifugation at 15000 rpm for 20 min, then divided into aliquots and stored at −80 °C until the time of the following biochemical investigations were performed: serum cholesterol, triglycerides, HDL-C and LDL-C were measured colorimetrically using kits that were provided by Abbott diagnostic. Alanine transaminase enzyme (ALT), aspartate transaminase enzyme (AST), alkaline phosphatase (ALP), and γ glutamyltranspeptidase (GGT) activities were estimated by enzymatic methods (Sigma–Aldrich).

Ray-Bio Rat insulin ELISA kit an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of rat insulin in serum. Fasting blood glucose was measured spectrophotometrically (AGAPPE Diagnostics Switzerland GmbH, Switzerland). In each sample, the degree of insulin resistance (IR) was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) as described by Matthews *et al.*[11] HOMA-IR was calculated according to the equation: fasting insulin (μU/ml) × fasting glucose (mmol/L)/22.5. Moreover, serum levels of SMP-30, 25 hydroxy-vitamin D3 and insulin were determined by using enzyme-linked immunosorbent assay using ELISA technique. Serum 25 hydroxy-vitamin D3 level was measured using Rat 25 hydroxy vitamin D_3 (25 (OH) D_3) ELISA kit -catalog number MBS261766-MyBioSource-Southern California, San Diego (USA). SMP-30 in rat serum was measured using Rat RGN/Regucalcin ELISA kit (Sandwich ELISA) - LS-F38749- LifeSpan Biosciences-USA.

Statistical Analysis

SPSS statistical software (version 20) was used for the statistical analysis of the collected data. The results were presented as means ± standard deviation (mean ± SD) or as frequencies according to variable types. Independent t-test was used for comparison between variables. One-way ANOVA test and the post-hoc test (Fisher's least significant difference) were used for multiple comparisons. Pearson correlation coefficient was used to make correlations between different variables.

RESULTS

Fifty adult male Sprague–Dawley albino rats were enrolled in the current study. Five rats died during the course of the experiment (four from NALFD model group and one from vitamin D–treated NAFLD group), and the total number of rats became 45 rats at the end of the study.

The anthropometric and the biochemical data of the studied groups are presented in Table 1. Total body weight and BMI of rats that are enrolled in the current study are higher in NAFLD models (nontreated and vitamin D treated) than the control groups (P<0.001) with no significant difference between the two NAFLD groups. While hepatic index is significantly lower in NAFLD groups than the control groups with no differences between them (P<0.001). Height of all studied rats shows nonsignificant differences (p=0.94).

Serum glucose, insulin, and HOMA-IR are significantly higher in NAFLD model group of rats than the control groups (P<0.001), but nonsignificant change in vitamin D–supplemented NAFLD group of rats.

Regarding lipid profile, total cholesterol, triglycerides, and LDL-C concentration are higher in NAFLD model group than the control groups (P<0.001) with significant decrease in vitamin D–supplemented-NAFLD disease than the nontreated group. HDL-C shows a significant decrease in NAFLD model group than the control groups (P<0.001) with significant increase in vitamin D–supplemented-NAFLD disease than the nontreated group.

Liver function tests show the following data: no significant difference between the studied groups in serum albumin and ALP (p=0.16 and 0.15, respectively). While AST, Alt and GGT are significantly higher in NAFLD groups than the control groups with no significant difference between the vitamin D–supplemented NAFLD and nontreated NAFLD group.

Serum 25(OH)D3 concentration is significantly lower in NAFLD model groups (nontreated and vitamin D treated) than the control groups (negative and positive control groups) (P<0.001). The vitamin D–supplemented NAFLD group shows higher serum level of 25(OH)D3 concentration than nonsupplemented NAFLD model.

Correlations of serum 25(OH)D3 (nmol/l) level with anthropometric and the biochemical parameters are presented in Table 2. There are significant negative correlations between serum level of 25(OH)D3 and all studied parameters in all studied rats (P<0.001) with the exception

Table 1 Anthropometric and Biochemical Parameters of All Studied Animal Groups.

	Group I negative control N = 10	Group II nontreated NAFLD group model N = 11	Group III positive control group N = 10	Group IV NAFLD + vitamin D supplement N = 14	ANOVA *P* value
Weight (gm)	194.80 ± 1.81	343.55 ± 5.80[a]	199.70 ± 2.68[b]	337.71 ± 3.72[a,c]	<0.001
Height (cm)	21.26 ± 0.45	21.49 ± 0.32	21.23 ± 0.26	21.26 ± 0.34	0.94
BMI (gm/cm^2)	0.43 ± 0.06	0.74 ± 0.07[c]	0.44 ± 0.03[a,b]	0.72 ± 0.07[a,b]	<0.001
Hepatic index	0.05 ± 0.001	0.04 ± 0.001[a]	0.05 ± 0.001[b]	0.04 ± 0.001[a,c]	<0.001
Insulin (μu/L)	25.54 ± 0.93	35.72 ± 0.92[a]	24.56 ± 1.69[b]	36.56 ± 1.10[a,c]	<0.001
Glucose (mmol/L)	5.28 ± 0.35	7.88 ± 0.28[a]	4.69 ± 0.26[b]	7.72 ± 0.22[a,c]	<0.001
HOMA-IR	0.33 ± 0.03	0.69 ± 0.03[a]	0.28 ± 0.03[b]	0.69 ± 0.03[a,c]	<0.001
T Chol. (mmol/L)	2.73 ± 0.23	4.71 ± 0.30[a]	2.82 ± 0.23[b]	4.59 ± 0.38[a,c]	<0.001
TG (mmol/L)	1.04 ± 0.08	1.33 ± 0.21[a]	1.00 ± 0.09[b]	1.23 ± 0.23[a,c]	0.001
LDL-C (mmol/L)	1.19 ± 0.17	2.34 ± 0.74[a]	1.00 ± 0.09[b]	1.72 ± 0.44[a,c]	0.001
HDL-C (mmol/L)	1.07 ± 0.10	1.53 ± 0.30[a]	1.01 ± 0.09[b]	1.89 ± 0.77[a,c]	<0.001
Albumin (g/dl)	3.51 ± 0.02	3.41 ± 0.04	3.46 ± 0.02	3.48 ± 0.02	0.16
AST (U/L)	44.75 ± 1.03	57.07 ± 0.82[a]	45.62 ± 0.73[b]	56.66 ± 1.18[a,c]	<0.001
ALT (U/L)	35.57 ± 0.76	46.34 ± 0.59[a]	35.41 ± 0.56[b]	45.57 ± 0.70[a,c]	<0.001
ALP (U/L)	67.64 ± 1.10	66.58 ± 1.79	70.45 ± 1.51	64.40 ± 1.66	0.15
GGT (U/L)	8.97 ± 0.13	13.55 ± 0.30[a]	9.25 ± 0.12[b]	13.00 ± 0.34[a,c]	<0.001
25 OHD3 (nmol/l)	13.24 ± 0.23	7.16 ± 0.37[a]	14.36 ± 0.36[b]	10.04 ± 0.26[a,b,c]	<0.001
SMP-30 (pg/ml)	117.20 ± 0.87	83.21 ± 0.81[a]	116.70 ± 1.19[b]	84.18 ± 0.76[a,c]	<0.001

Data are presented as mean ± standard deviation.
Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc analysis.
[a]Significantly different from N.C value at P < 0.05.
[b]Significantly different from NAFLD value at P < 0.05.
[c]Significantly different from N.C + Vit D value at P < 0.05.

Table 2 Correlation of 25 Hydroxycholecalciferol (nmol/l) Level with Anthropometric and the Biochemical Parameters.

	All the studied population		Group I negative control N = 10		Group II nontreated NAFLD group model N = 11		Group III positive control group N = 10		Group IV NAFLD + vitamin D supplement N = 14	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Weight (gm)	−0.85	<0.001	−0.24	0.51	−0.30	0.29	−0.23	0.53	0.13	0.71
Height (cm)	−0.13	0.60	0.19	0.60	−0.25	0.39	−0.48	0.17	0.04	0.81
BMI (gm/cm^2)	−0.75	<0.001	−0.25	0.48	0.09	0.77	0.21	0.41	0.02	0.94
Hepatic index	0.85	<0.001	0.43	0.21	0.53	0.05	0.02	0.95	0.12	0.72
Insulin (μu/L)	−0.73	<0.001	0.15	0.68	0.21	0.21	−0.40	0.25	−0.51	0.05
Glucose (mmol/L)	−0.71	<0.001	0.64	0.05	0.26	0.38	0.05	0.89	−0.23	0.41
HOMA-IR	−0.76	<0.001	0.57	0.09	0.34	0.23	−0.30	0.39	−0.52	0.01
T Chol. (mmol/L)	−0.84	<0.001	0.06	0.87	−0.12	0.68	0.41	0.14	−0.06	0.87
TG (mmol/L)	−0.51	<0.001	−0.23	0.53	0.23	0.42	0.20	0.58	−0.04	0.97
LDL-C (mmol/L)	−0.59	<0.001	−0.46	0.18	−0.70	0.00	−0.26	0.47	−0.35	0.32
HDL-C (mmol/L)	−0.57	<0.001	0.13	0.72	0.12	0.78	−0.31	0.38	−0.18	0.62
Albumin (g/dl)	0.16	0.21	−0.67	0.03	−0.38	0.18	0.47	0.18	−0.10	0.76
AST (U/L)	−0.74	<0.001	0.15	0.68	0.48	0.08	0.25	0.48	−0.31	0.36
ALT (U/L)	−0.79	<0.001	0.29	0.42	0.26	0.37	0.12	0.75	0.37	0.27
ALP (U/L)	0.25	0.01	0.13	0.72	0.19	0.52	−0.19	0.51	0.23	0.41
GGT (U/L)	−0.83	<0.001	−0.51	0.13	0.23	0.43	−0.01	0.99	−0.46	0.15

Statistical analysis was carried out using Spearman's correlation analysis. r = Spearman's rank correlation coefficient, **P*-value ≤0.05 is considered significant.

of hepatic index (positive correlation r=0.85 and *P*<0.001). While nonsignificant correlations were found with height, serum albumin, and ALP activity (r = −0.13, 0.16, and 0.25; p=0.60, 0.30, and 0.10, respectively). No significant correlation was found in the studied individual groups. Table (3) shows the correlation between serum SMP-30 (pg/ml) level and the studied parameters. There are significant negative correlations between serum level of SMP-30 and all studied parameters in all studied rats (*P*<0.001) with the exception of hepatic index (positive correlation r=0.94 and *P*<0.001). While nonsignificant correlations were found with height, serum albumin, and ALP activity (r = −0.10, 0.16, and 0.26; p=0.53, −0.30, and 0.08, respectively). No significant correlation was found in the studied individual groups.

A significant positive correlation between serum 25(OH)D3 (nmol/l) and SMP-30 (pg/ml) in all studied groups (r=0.87 and *P*<0.001). No other correlation in the other individual studied groups as shown in Table 4.

DISCUSSION

In the current study, there are increased BMI, glycemic and IR parameters in NAFLD model with decreased hepatic index. These findings indicate the intimate association between IR and NAFLD pathogenesis. IR has been proved to be associated with NAFLD. Gutierrez-Buey *et al.*[12] reported that high HOMA-IR is independently associated with the presence of NAFLD.This is in accordance with a study previously conducted by Targher G *et al*[13]

Observational studies had shown a direct correlation between plasma adiponectin concentrations and adiposity, IR, and hepatic fat.[14,15] Ko *et al.*[16] reported that 96% of patients with NAFLD exhibited IR with HOMA-IR>2 in their study.

NAFLD is intimately linked to insulin resilience in its pathogenesis.[17] NAFLD is associated with reduced body sensitivity to insulin, making it hard for such patients to regulate their blood glucose.[18] The main metabolic disorder that leads to lipid accumulation could be attributed to IR state. This could predispose to disturbed hepatic synthesis, secretion and degradation of lipid. Consequently, NAFLD is the result. So, IR is the most reproducible theory in triggering NAFLD.[19]

It is often thought that excessive secretion of insulin results from heredity, drugs, diabetes, obesity, lipid metabolism diseases, and other factors causing IR. It leads to an increased body fat content and mitochondrial dysfunction in liver cells that ultimately caused the first hit. Then, the liver cell degenerates into fat, reduces the cell viability, and increases the intracellular oxidative metabolites, which eventually causes the second hit, leading to the hepatic cell oxidative stress. Finally, in liver cells a series of

Table 3 Correlation of SMP-30 (pg/ml) Level with Anthropometric and The Biochemical Parameters.

	All the studied population		Group I negative control N = 10		Group II nontreated NAFLD group model N = 11		Group III ositive control group N = 10		Group IV NAFLD + vitamin D supplement N = 14	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Weight (gm)	−0.97	<0.001	0.47	0.17	−0.22	0.44	0.55	0.10	−0.04	0.91
Height (cm)	−0.01	0.53	−0.29	0.42	−0.44	0.12	−0.19	0.61	0.36	0.28
BMI (gm/cm^2)	−0.87	<0.001	0.35	0.32	0.33	0.26	0.31	0.26	−0.30	0.36
Hepatic index	0.94	<0.001	−0.19	0.51	0.32	0.27	−0.28	0.43	−0.03	0.93
Insulin (μu/L)	−0.79	<0.001	−0.08	0.83	−0.17	0.57	0.66	0.04	0.34	0.30
Glucose (mmol/L)	−0.82	<0.001	−0.26	0.47	0.09	0.75	0.48	0.17	−0.51	0.11
HOMA-IR	−0.86	<0.001	−0.19	0.61	−0.02	0.95	0.87	0.001	−0.08	0.81
T Chol. (mmol/L)	−0.94	<0.001	0.06	0.87	−0.15	0.68	0.32	0.37	0.17	0.65
TG (mmol/L)	−0.57	<0.001	−0.23	0.53	0.23	0.42	−0.20	0.58	−0.16	0.67
LDL-C (mmol/L)	−0.63	<0.001	0.46	0.18	−0.17	0.55	−0.08	0.83	0.25	0.44
HDL-C (mmol/L)	−0.59	<0.001	0.13	0.72	−0.12	0.68	0.41	0.25	0.21	0.40
Albumin (g/dl)	0.16	−0.30	0.31	0.26	−0.21	0.30	−0.14	0.71	−0.19	0.58
AST (U/L)	−0.89	<0.001	−0.04	0.91	0.01	0.74	−0.24	0.51	−0.56	0.07
ALT (U/L)	−0.91	<0.001	0.34	0.35	−0.04	0.89	0.16	0.67	−0.28	0.40
ALP (U/L)	0.26	0.08	−0.20	0.57	−0.43	0.13	0.27	0.46	0.36	0.28
GGT (U/L)	−0.91	<0.001	0.57	0.09	0.12	0.61	−0.01	0.98	−0.16	064

Statistical analysis was carried out using Spearman's correlation analysis. r = Spearman's rank correlation coefficient, *P-value ≤0.05 is considered significant.

inflammatory responses, necrosis, and fibrosis happen.[20–22] It is called two-hit hypothesis of NAFLD pathogenesis.

Recently, the theory that is commonly adopted is the "multihit model," which involves interaction of genetic and environmental factors.[23,24] Moreover, mitochondrial dysfunction and endoplasmic reticulum stress-associated mechanisms share in the pathogenesis of NAFLD.[25,23]

The present study shows a significant increase in total cholesterol, LDL-C and TG levels as with a significant decrease in HDL-C level in NAFLD model group in comparison with the other groups of the study. This finding proved that NAFLD is associated with dyslipidemia. These result are in accordance to a study by Mahaling *et al.*[26] and Deeb *et al.*[27] They found that serum total cholesterol, serum HDL-C, LDL-C, and VLDL show statistical significant increase with higher grades of NAFLD. A recent study conducted by Amor and Perea[28] discussed the relation between NAFLD and dyslipidemia. They reported lipoprotein derangements in NAFLD. They found an increased number of very low-density lipoprotein, small-dense low-density lipoprotein, and triglyceride contents in lipoprotein particles. Moreover, there are smaller high-density lipoprotein particles with impaired function. Finally, they concluded that the dominance of triglyceride-rich lipoproteins is suggested to be the key prominent lipoprotein disorder in NAFLD.[28]

Regarding liver function tests in the current study, there is mild elevation in liver enzymes AST, ALT, and GGT with nonsignificant change in serum albumin and ALP. Our results confirmed the results of Sanyal *et al.*[29] They reported nearly a similar result. So, ALT, AST, and GGT are not useful in predicting or monitoring the state of NAFLD [29].

A prominent finding of the current study is serum 25(OH)D3 is lower in NAFLD model group in comparison with the control groups. It is suggested that low serum 25(OH)D3 may cause metabolic diseases[30] and NAFLD.

Table 4 Correlation Between 25 Hydroxycholecalciferol and SMP-30 in All Studied Groups.

	All the studied population		Group I negative control N = 10		Group II nontreated NAFLD group model N = 11		Group III positive control group N = 10		Group IV NAFLD + vitamin D supplement N = 14	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
SMP-30	0.87	<0.001	−0.21	0.40	0.54	0.05	−0.21	0.57	0.09	0.71

Statistical analysis was carried out using Spearman's correlation analysis. r = Spearman's rank correlation coefficient, *P-value ≤0.05 is considered significant.

Hypovitaminosis D is correlated with the incidence and severity of NAFLD.[31] Recently, it reported that NAFLD is associated with 26% higher risk of vitamin D deficiency in a recent meta-analysis by Hariri and Zohdi.[32]

The relationship between obesity and inadequacy of vitamin D had been discussed by González-Molero[33] and Cordeiro.[34] The synergistic interaction between them increased the risk of IR. There is an inverse correlation between serum of 25(OH)D3 concentrations and higher measures of obesity, including BMI (≥30 kg/m2), high fat mass, and waist circumference.[35] It is reported that each unit increase in BMI is being associated with 1.15% lower concentration of 25(OH)D3.[36] This is in accordance with previous study that suggested that deficiency of vitamin D is a significant risk factor for the development of NAFLD[37] and indicate that the anti-inflammatory and immune modulatory ability of vitamin D may provide credible mechanisms by which vitamin D may enhance NAFLD progression and severity. Another study from China found that vitamin D can stimulate endogenous fatty acid biosynthesis in NASH through a mechanism that involve impairment of enterohepatic circulation, and this is proved by that the administration of vitamin D corrected NASH in correspondence with suppressed inflammation and hepatic lipogenesis.[38]

Regarding cellular senescence, the current study shows that NAFLD group possesses a significant decrease in SMP-30 level compared with normal controls. This result is in an agreement with Schafer *et al,*[39] Ogrodnik *et al.*[5] and Papatheodoridi *et al.*[40]

A direct relationship between the markers of cellular senescence and fat accumulation in hepatocytes of mice and in NAFLD patients had been documented by Ogrodnik *et al.*[5] and their results are confirmed in a recent study published by Papatheodoridi *et al.*[40] The SMP-30, is an antioxidant and anti-apoptotic protein, and its expression decreases with aging. The repressed expression of SMP-30 is linked to the development of NAFLD.[41]

The progression of liver disease was affected by cellular senescence and telomere dysfunction.[42] A significant inducer of hepatocyte senescence has been linked to impairment of liver regeneration.[43] Mitochondrial dysfunction and consequent oxidative stress are also considered as evident triggers for cellular senescence in NAFLD. This is a regulated by signaling through p21 and p38 mitogen-activated protein kinases[42] and deregulation of the mammalian target of rapamycin pathway.[44] This mitochondrial dysfunction is a characteristic of both aging and IR-related disorders.[20] Furthermore, mitochondrial-induced reactive oxygen species is implicated in telomere dysfunction, consequently contributing to cellular senescence.[42]

The evident protective role of vitamin D supplementation is observed in the current study in the form of improvement of the biochemical parameters in vitamin D–supplemented NAFLD group. These findings come in agreement with Nakano *et al,*[45] Sakpal *et al.*[46]; Liu *et al.*[47] Sakpal *et al.*[46] found that vitamin D supplementation plays a role in the treatment of patients with NAFLD along with lifestyle modifications in their human study. They proved the therapeutic role of vitamin D on NAFLD in their study by significant rise of adiponectin and decrease in TNF-α in vitamin D–supplemented group in comparison with others in their study. In contrast, our findings are critically discussed recently by Sharifi and Amani.[48] They put some recommendations to be considered to follow and judge before start to use vitamin D supplement for prevention or treatment of NAFLD. These considerations include gender differences, baseline serum status of vitamin D, co-supplementation with calcium, and gene polymorphism.[48]

The pivotal protective role of vitamin D against NAFLD development could be explained by the role of vitamin D receptor (VDR). VDRs are expressed in hepatic cells, and its expression can suppress inflammation in chronic hepatic diseases.[31] In vitro studies it is reported that VDR could enhance of glucose transporter-4 muscular expression, modulate free fatty acids and consequently increased insulin sensitivity.[49] Moreover, vitamin D has antifibrotic, antiproliferative, and anti-inflammatory effects on the liver. Furthermore, vitamin D reduces expression of cytokeratin 18 apoptotic fragment M30,[50] which acts as a marker of hepatic damage. Moreover, Liu *et al.*[47] add additional mechanism of the potential protective role of vitamin D supplementation in slowing the rate of NAFLD development via suppression of the p53 pathway, thus preventing the progression of NAFLD. So, the active vitamin D supplementation could potentially hinder hepatocyte senescence. This is confirmed in the current study by the evident increased SMP-30 level. Consequently, our and Lui *et al.*[47] studies provide an evidence on the potential protective role of vitamin D supplementation in NAFLD.

From the current study, it could be concluded that vitamin D deficiency is a prominent finding in NAFLD and may have a role in its pathogenesis through enhancement of cellular senescence in addition to its potential role in increasing IR. Vitamin D supplementation plays a protective role, which needs further investigation at the molecular and gene expression level using larger number of samples. Moreover, clinical study is also recommended.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Hasen A. Al-ghamdi: collected, Formal analysis, interpreted the data, Writing - review & editing, approved the final manuscript. **Fayza F. Al Fayez:** collected, Formal analysis, interpreted the data, Writing - review & editing, approved the final manuscript. **Abdulhadi I. Bima:** collected, Formal analysis, interpreted the data, Writing - review & editing, approved the final manuscript. **Taghreed**

M. Khawaji: collected, Formal analysis, interpreted the data, Writing - review & editing, approved the final manuscript. **Ayman Z. Elsamanoudy:** conceived, Methodology, Writing - original draft.

CONFLICTS OF INTEREST

The authors have none to declare.

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