Hepatic Steatosis and Diabetes Mellitus: Risk Factors, Pathophysiology and with its Clinical Implications: A Hospital Based Case Control Study in Western Region of Nepal

Mittal A[1], Sathian B[2], Chandrasekharan N[3], Lekhi A[4], Rahib R[5] , Dwedi S[6]

[1]Associate Professor, Department of Biochemistry, Manipal College of Medical Sciences, Pokhara, Nepal.

[2]Assistant Professor, Department of Community Medicine, Manipal College of Medical Sciences, Pokhara, Nepal.

[3]Assistant Professor, Department of Orthopaedics, Manipal College of Medical Sciences, Pokhara, Nepal.

[4]MBBS Intern, Manipal College of Medical Sciences, Pokhara, Nepal.

[5]Final year MBBS Student, Manipal College of Medical Sciences, Pokhara, Nepal.

[6]Laboratory Technologist, Department of Biochemistry, Manipal College of Medical Sciences, Pokhara, Nepal.

Original Article

Corresponding Author:
Dr. Ankush Mittal, Associate Professor,
Department of Biochemistry, Manipal College of Medical Sciences, Pokhara, Nepal.
Email: drmittala@gmail.com

Abstract

Background

The perception of nonalcoholic fatty liver disease (NAFLD) as an infrequent and benign condition is swiftly altering in developing countries as there has been an upsurge in non alcoholic fatty liver disease in Asia-Pacific region. NAFLD develops across all age groups and societies and is recognized to occur in 14%–30% of the common population. The foremost risk factors for NAFLD such as central obesity, diabetes mellitus, insulin resistance, dyslipidemia, hypertension, hypertriglyceridemia are currently predominant and puts a very large population at risk of evolving hepatic steatosis in the coming decades.

Material and Methods

It was a hospital based case control study carried out in the Department of Biochemistry of Manipal Teaching Hospital, Pokhara, Nepal between 1st January 2010 and 31st Dec 2010. The variables collected were age, gender, fasting blood glucose, total cholesterol, low density lipoproteins, triglycerides, high density lipoproteins, very low density lipoproteins, aspartate transaminase, alanine transaminase.

Results

Of the 200 patients of non alcoholic fatty liver disease patients with diabetes mellitus, all the variables except triglycerides shows insignificant disparity in relation to gender. The perceptible difference was observed in mean values of triglycerides for cases of NALFD between diabetes (218.25 ± SD 73.68) and non diabetic subjects (177.54 ± SD73.45) (p=.0001). The mean values of HDL did not illustrate much difference in cases of NALFD with diabetes (41.54 ± SD2.13) and non diabetic subjects (44.24 ± SD2.05).

Conclusion

Public health initiatives are undoubtedly of the essence to halt or turn around the global 'diabesity' pandemic, the causal basis of NAFLD. Management of patients with NAFLD should be aimed at treating metabolic risk factors

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such as hyperglycemia and hypertriglyceridemia. Successful lifestyle adaptation with increased exercise and decreased food intake is able to remove the accumulation of liver fat and can reverse insulin resistance.

Keywords
Hepatic steatosis, Diabetes mellitus, Risk factors, Nepal

Background

The perception of nonalcoholic fatty liver disease (NAFLD) as an infrequent and benign condition is swiftly altering in developing countries as there has been an upsurge in non alcoholic fatty liver disease in Asia-Pacific region. NAFLD transpires across all age groups and societies and is recognized to occur in 14%–30% of the common population[1] . The foremost risk factors for NAFLD such as central obesity, diabetes mellitus, insulin resistance dyslipidemia, hypertension and hypertriglyceridemia currently prevail and puts a very large population at risk of evolving and progression of hepatic steatosis in the coming decades. Pathogenetic insight of NAFLD include overnutrition, insulin resistance (IR) and genetic aspects[2]. NAFLD and type 2 diabetes mellitus recurrently coincide as both contribute to the pathogenic abnormalities of excess adiposity and insulin resistance[3]. Roughly 70% of persons with non insulin dependent diabetes mellitus have a fatty liver and the disease trails a more aggressive course with necro-inflammation and fibrosis in diabetes[4]. Non-insulin-dependent diabetes mellitus is a multifaceted metabolic disorder that involves numerous biochemical abnormalities, a heterogeneous clinical picture, and a polygenic genetic module. The pathophysiologic state of non insulin dependent diabetes mellitus encompasses increased basal hepatic glucose production, decreased insulin-mediated glucose consumption in target tissues and reformed pancreatic function with decreased beta cell function and enhanced glucagon secretion. In non-insulin dependent diabetes mellitus, there is compromised autophosphorylation-kinase activity of insulin receptors when sequestered from adipocytes, liver, erythrocytes and skeletal muscles, leading to insulin resistance[5]. Insulin resistance plays a vital role in NAFLD pathogenesis. Insulin resistance is often allied with chronic low-grade inflammation, and several mediators released from immune cells and adipocytes may lead to hepatocellular injury and liver disease progression[6]. Nonalcoholic steatohepatitis(NASH), a histological subtype of NAFLD characterized by hepatocyte injury and inflammation is present in approximately 10% of patients with non-insulin diabetes mellitus and can progress to cirrhosis and liver failure. It is estimated to be the most common cause of cryptogenic cirrhosis at present[7]. Diabetes mellitus has a significant role in worsening of fibrosis. Overproduction of glucose, very low-density lipoproteins, triglycerides, C-reactive protein and coagulation factors by the fatty liver may perhaps add to the excess risk of cardiovascular disease. Lack of knowledge regarding disease status, risk factors and reduced level of prior diagnosis leads to the high prevalence and mortality due to diabetes and NALFD among Nepalese population[8]. The objective of our study was concerned primarily to correlate foremost risk factors involved in the pathogenesis of hepatic steatosis, in order to prevent the succession of complications in Pokhara valley.

Materials and Methods

It was a hospital based case control study carried out in the Department of Biochemistry of Manipal Teaching Hospital, Pokhara, Nepal between 1st January 2010 and 31st December 2010. The variables collected were age, gender, fasting blood glucose, total cholesterol, low density lipoproteins, triglycerides, high density lipoproteins, very low density lipoproteins, aspartate transaminase and alanine transaminase. Approval for the study was obtained from the institutional research ethical committee.

Estimation of blood glucose was done by glucose oxidase and peroxidase method[9]. Estimation of total cholesterol and triglycerides was done by CHOD-PAP and GPO-PAP method respectively[10]. Estimation of high density lipoproteins was done by kinetic enzymatic method[11]. The values of low-density lipoprotein cholesterol (LDL) and triglycerides were obtained by the Friedewald formula[12]. The transaminases (AST and ALT) were estimated by liqui uv test[13]. All these laboratory parameters were analysed using Human reagent kits and with the help of semi autoanalyser (Human, Germany). Analysis was done using descriptive statistics and testing of hypothesis. The data was analyzed using Excel 2003, R 2.8.0 Statistical Package for the Social Sciences (SPSS) for Windows Version 16.0 (SPSS Inc; Chicago, IL, USA) and the EPI Info 3.5.1 Windows Version. The Chi-square test was used to examine the association between different variables. Z-test was used to compare the significance difference between two variables. A p-value of < 0.05 (two-tailed) was used to establish statistical significance.

Selection of Subjects:

Inclusion criteria: 200 patients with Diabetes Mellitus who sought treatment for diabetes at the endocrinology unit of Medicine Department in Manipal Teaching Hospital between 1st January 2010 and 31st Dec 2010 were evaluated. Subjects were subsequently divided into groups of normal glucose (NG) and DM. According to the American Diabetes Association guidelines, subjects with normal fasting glucose had values below 100 mg/dL and subjects with DM were defined by fasting glucose above 126 mg/dL[14]. 200 DM patients were compared with a control group of 200 healthy adults with no family history of diabetes. Healthy controls were volunteers employed at Manipal Teaching Hospital, Pokhara. Evidence of fatty liver was obtained by performing ultrasound of the abdomen. Ultrasound demonstrating diffusely increased liver echogenicity with blurring of the intrahepatic vessels and diaphragm, or bright hepatic echogenicity with poor penetration of the posterior hepatic segments and intrahepatic vessels with indication of contrast between the liver and kidney confirms the diagnosis of non alcoholic

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fatty liver disease[15].

Exclusion criteria: Subjects also were excluded from the diagnosis of NAFLD when they were having extreme alcohol ingestion (women: ≥20 g/wk, men: ≥30 g/wk), positive hepatitis B surface antigen (HBsAg) or anti-hepatitis C virus antibody (anti-HCV), pregnancy, entire parental nutrition, jejuneal bypass or extensive small bowel resection, or other known liver diseases like hepatoma, as determined by history, physical examination and screenisng blood tests. Subjects who had ingested drugs known to produce fatty liver disease such as steroids, estrogens, amiodarone, tamoxifen or other chemotherapeutic agents within the previous 6 months were also excluded from our present study.

Results

Of the 200 cases of NALFD, there was the perceptible difference in mean values of triglycerides between diabetes and non diabetic subjects. Of the 200 cases of DM, the moderate difference was perceived in mean values of triglycerides with NALFD and no NALFD patients.

Table 1: Gender wise comparison of non alcoholic fatty liver disease patients with diabetes mellitus cases

Variables	Female(60)		Male(140)		p value
	Mean values ± S D	Confidence Interval	Mean values ± S D	Confidence Interval	
Age	52.85± 14.66	(49.06,56.64)	52.49± 14.78	(49.97,55.00)	0 .874
FBS	162.45 ± 48.49	(149.92,174.98)	158.65± 42.05	(151.50,165.80)	0.600
TC	205.52 ± 43.26	(194.34,216.69)	202.08± 43.16	(194.73,209.43)	0.610
TG	204.17± 65.06	(187.36,220.97)	224.51± 76.60	(211.47,237.55)	0.590
HDL	41.82 ± 2.08	(41.28,42.36)	41.41± 2.15	(41.05,41.78)	0.220
LDL	124.25 ± 35.50	(115.08,133.4)	113.17± 38.83	(106.56,119.78)	0.053
VLDL	40.80 ± 12.87	(37.47,44.13)	44.63 ± 15.28	(42.04,47.22)	0.073
AST	29.87 ± 3.96	(28.84,30.89)	28.83± 3.38	(28.25,29.41)	0.081
ALT	31.85 ± 4.56	(30.67,33.03)	31.87± 5.02	(31.02,32.37)	0 .974

* Statistically significant (p<0.05)

Table 1 depicts that of the 200 patients of non alcoholic fatty liver disease patients with diabetes mellitus, all the variables except triglycerides shows insignificant disparity in relation to gender. The mean values of triglycerides were more in males (224.51 ± SD76.60) when compared to females (204.17 ± SD65.06) and above the upper limit of normal reference range.

Table 2: Comparison of variables for cases of diabetes mellitus with NALFD and with no NALFD patients

Variables	NALFD(-)200		NALFD(+)200		p value
	Mean values ± S D	Confidence Interval	Mean values ± S D	Confidence Interval	
Age	51.98 ± 14.31	(49.9,53.9)	52.60 ± 14.71	(50.52,54.68)	0.669
FBS	160.40 ± 44.62	(154.2,166.6)	159.82 ± 44.02	(153.6,166.1)	0.890
TC	193.28 ± 40.60	(187.6,198.9)	203.14 ± 43.11	(197.1,209.2)	0.020*
TG	196.56 ± 73.40	(186.3,206.79)	218.25 ± 73.68	(207.8,228.6)	0.004*
HDL	42.40 ± 2.06	(42.12,42.69)	41.54 ± 2.13	(41.24,41.84)	0 .0001*
LDL	110.98 ± 38.03	(105.7,116.28)	116.58 ± 38.09	(111.2,121.9)	0.145
VLDL	39.15 ± 14.52	(37.12,41.18)	43.45 ± 14.62	(41.39,45.52)	0.004*
AST	26.09 ± 3.58	(25.59,26.59)	29.15 ± 3.59	(28.64,29.66)	0.0001*
ALT	27.82 ± 4.83	(27.14,28.49)	31.87 ±4.87	(31.18,32.55)	0.0001*

* Statistically significant (p<0.05)

Table 2 illustrates the differences in distribution of variables for cases of DM with NALFD and no NALFD patients. There was insignificant difference in mean values of fasting blood sugar for cases of DM with NALFD (159.82 ± SD44.02) and no NALFD patients (160.40 ± SD 44.62) (p=0.890). There was mild discrepancy in mean values of total cholesterol with NALFD (203.14 ± SD43.11) and no NALFD patients (193.28 ± SD40.60) (p=.020). The moderate difference was perceived in mean values of triglycerides for cases of DM with NALFD (218.25 ± SD73.68) and no NALFD patients (196.56 ± SD73.40) (p=.004). The mean values of HDL did not exemplify much difference in cases of diabetes mellitus with NALFD (41.54 ± SD2.13) and no NALFD subjects (42.40 ± SD2.06) (p= .0001). There was mild difference for the mean values of LDL for cases of DM with NALFD (116.58 ± SD38.09) and no NALFD patients (110.98 ± SD38.03) (p=

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0.145) but well below the upper limit of normal reference range. The mean values of alanine and aspartate transaminases were in normal range in diabetes mellitus patients with NALFD or without NALFD.

Table 3: Comparison of variables for cases of NALFD with DM patients and subjects with no family history of diabetes

Variables	DM(-)(200)		DM(+)(200)		p value
	Mean values ± S D	Confidence Interval	Mean values ± S D	Confidence Interval	
Age	54.42 ± 12.02	(50.74,54.10)	52.60 ± 14.71	(50.52,54.68)	0.918
FBS	89.66 ± 10.66	(88.17,91.15)	159.82 ± 44.01	(153.60,166.04)	0.0001*
TC	183.43 ± 40.66	(177.7,189.11)	203.14 ± 43.11	(197.05,209.23)	0.0001*
TG	177.54 ±73.45	(167.3,187.81)	218.25 ±73.68	(207.84,228.66)	0.0001*
HDL	44.24 ± 2.05	(43.95,44.52)	41.54 ± 2.13	(41.24,41.84)	0.0001*
LDL	99.03 ± 38.11	(93.70,104.3)	116.58 ± 38.09	(111.20,121.96)	0.0001*
VLDL	35.60 ± 14.69	(33.54,37.65)	43.45 ± 14.62	(41.39,45.52)	0.0001*
AST	25.44 ± 3.57	(24.94,25.94)	29.15 ± 3.59	(28.64,29.66)	0.0001*
ALT	26.02 ± 3.61	(25.51,26.52)	31.87 ±4.87	(31.18,32.55)	0.0001*

* Statistically significant (p<0.05)

Table 3 depicts the differences in distribution of variables for cases of NALFD with DM patients and subjects with no family history of diabetes. **T**here was significant difference in mean values of fasting blood sugar between diabetes (159.82 ± SD44.01) and non diabetic subjects (89.66 ± SD10.66) (p=.0001) for cases of NALFD. There was slight variation in mean values of total cholesterol for cases of NALFD with diabetes (203.14 ± SD43.11) and non diabetic subjects (183.43 ± SD40.66) (p=.0001). The perceptible difference was observed in mean values of triglycerides for cases of NALFD between diabetes (218.25 ± SD 73.68) and non diabetic subjects (177.54 ± SD73.45) (p=.0001). The mean values of HDL did not illustrate much difference in cases of NALFD with diabetes (41.54 ± SD2.13) and non diabetic subjects (44.24 ± SD2.05). There was mild difference for the mean values of LDL difference in cases of NALFD with diabetes (116.58± SD38.09) and non diabetic subjects (99.03 ± SD38.11) but well within normal reference range. The mean values of AST did not show much variation in non alcoholic fatty liver disease patients with (29.15 ± SD3.59) or without (25.44 ± SD3.57) diabetes mellitus. Similarly, the mean values of ALT were also in the normal reference range in cases of NALFD with diabetes and non diabetic subjects.

Table 4: Comparison of variables for controls of NALFD with DM patients and subjects with no family history of diabetes

Variables	DM(-)(200)		DM(+)(200)		p value
	Mean values ± S D	Confidence Interval	Mean values ± S D	Confidence Interval	
Age	53.26 ± 16.14	(51.0,55.51)	51.98 ± 14.31	(49.98,53.97)	0.402
FBS	88.18 ± 11.92	(86.52,89.84)	160.40 ± 44.62	(154.17,166.6)	0.0001*
TC	172.00 ± 30.55	(167.7,176.25)	193.28 ± 40.60	(187.6,198.95)	0.0001*
TG	117 ± 24.62	(113.6,120.43)	196.56 ± 73.40	(186.3,206.79)	0.0001*
HDL	42.94 ± 2.01	(42.66,43.23)	42.40 ± 2.062	(42.12,42.69)	0.008*
LDL	103.52 ± 24.85	(100.1,106.99)	110.98 ± 38.03	(105.7,116.28)	0.021*
VLDL	23.36 ± 4.18	(22.70,24.03)	39.15 ± 14.52	(37.12,41.18)	0.0001*
AST	23.92 ± 3.39	(23.44,24.39)	26.09 ± 3.58	(25.59,26.59)	0.0001*
ALT	26.29 ± 4.19	(25.70, 26.88)	27.82 ± 4.85	(27.14,28.49)	0 .0001*

* Statistically significant (p<0.05)

Table 4 depicts the differences in distribution of variables for controls of NALFD with DM patients and subjects with no family history of diabetes. There was significant difference in mean values of fasting blood sugar for controls of NALFD with diabetes (160.40 ± SD44.62) and non diabetic subjects (88.18 ± SD11.92)(p=.0001). There was mild difference in mean values of total cholesterol for controls of NALFD with diabetes (193.28 ± SD40.60) and non diabetic subjects (172.00 ± SD30.55) (p=.0001). The discernible variation was observed in mean values of triglycerides for controls of NALFD with diabetes (196.56 ± SD73.40) and non diabetic subjects (117 ± SD24.62) (p=.0001). The other variables did not show much difference in mean values for controls of NALFD with diabetes and non diabetic subjects.

Discussion

NAFLD encompasses a spectrum of pathologic liver diseases ranging from simple hepatic steatosis to a predominant lobular necro-inflammation, with or without centrilobular

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fibrosis (called nonalcoholic steatohepatitis or NASH). NASH can progress to cirrhosis, decompensated liver disease, and hepatocellular carcinoma[16]. Of the 200 patients of non alcoholic fatty liver disease patients with diabetes mellitus, all the variables except triglycerides showed insignificant disparity in relation to gender. The mean values of triglycerides were more in males (224.51 ± SD76.60) (CI 211.47, 237.55) when compared to females (204.17 ± SD65.06) (CI 187.36, 220.97) and above the upper limit of normal reference range. The previous studies of NAFLD also indicated that men were more commonly affected than women[17]. Liver is a key site of action of insulin. Insulin resistance is a reproducible pathogenic factor in NAFLD pathogenesis and seems to be a common link between fatty liver and non insulin dependent diabetes mellitus[18]. The pathogenesis of type 2 diabetes involves both insulin resistance and defects in insulin secretion. Insulin resistance is an impairment of the physiological effects of insulin on concentration glucose. Regular glycemic control necessitates the pancreatic β cell sensing of glucose, production and liberation of insulin, binding of insulin to receptors with a subsequent commencement of a number of signaling proteins[19]. The activation of multiple signaling cascades cause increased glucose uptake by muscles and liver and decreased glucose production by the liver[20]. These molecular mechanisms are distorted in non insulin dependent diabetes mellitus causing insulin resistance in muscle tissue and increased hepatic glucose output[21]. The present study demonstrate that there was significant difference in mean values of fasting blood sugar for controls of NALFD with diabetes (160.40 ± SD44.62) (CI 154.17, 166.62) and non diabetic subjects (88.18 ± SD11.92) (CI86.52, 89.84) (p=.0001). When hyperglycemia supervenes, both insulin secretion and insulin-mediated glucose utilization are further compromised, mediated in part by sustained hyperglycemia itself. Furthermore, it results in insulin resistance and elevated plasma insulin concentrations. Several mechanisms have been hypothesized to cause TG abnormalities in type 2 diabetes subjects. Elevated plasma insulin concentrations enhance VLDL synthesis leading to hypertriglyceridemia[22]. Dyslipidemia, mainly hypertriglyceridemia, increase VLDL and decrease in HDL levels that accompanies type 2 diabetes plays an important role in the pathogenesis of accelerated atherosclerosis in this population. The current study revealed that the discernible variation was observed in mean values of triglycerides for controls of NALFD with diabetes (196.56 ± SD73.40) (CI186.33, 206.79) and non diabetic subjects (117 ± SD24.62) (CI 86.52, 89.84) (p=.0001). Diabetic patients illustrate delayed triglyceride clearance from plasma as compared to controls[23]. In hepatocytes, insulin resistance is related to hyperglycaemia and hyperinsulinaemia, formation of advanced glycation end-products, increased free fatty acids and their metabolites, oxidative stress and altered profiles of adipocytokines[2]. Accumulation of triglycerides could result in fatty liver because of increased uptake of free fatty acids and de novo synthesis exceeds hepatic lipid export and utilization by hepatocytes, which could potentially result from insulin resistance and alterations in lipid metabolism. The current study showed that there was the perceptible difference in mean values of triglycerides for cases of NALFD between diabetes (218.25 ± SD 73.68) (CI 207.84, 228.66) and non diabetic subjects (177.54 ± SD73.45) (CI 167.28,187.81)(p=.0001). Dyslipidemias are factors commonly associated with NAFLD. Previous studies which concurred with the findings of our results have shown that 20-92% of patients diagnosed with NAFLD have hyperlipidemia, mainly hypertriglyceridemia[24]. An ultrasound finding done by Assy et al also suggest that 50% of patients have hypergtriglyceridemia for detected fatty infiltration of the liver[25]. Excess of free fatty acids are oxidized and generates reactive oxygen species. The fatty liver is vulnerable to hepatocellular injury initiated by reactive oxygen species (ROS). ROS, increased intrahepatic levels of fatty acids, adipocytokines, tumor necrosis factor-α mitochondrial dysfunction, vascular disturbance provide a source of oxidative stress with subsequent lipid peroxidation and cytokine induction are precipitating factors in the cascade of events leading from simple steatosis to NASH[26]. The current study revealed that the mean values of HDL was decreased in cases of NALFD with diabetes (41.54 ± SD2.13) (CI 41.24, 41.84) and non diabetic subjects (44.24 ± SD2.05) (CI 43.95, 44.52). There was mild difference for the mean values of LDL difference in cases of NALFD with diabetes (116.58 ± SD38.09) (CI 111.20, 121.96) and non diabetic subjects (99.03 ± SD38.11) (CI 93.70, 104.36). NALFD leads to lowering of high-density lipoprotein cholesterol and formation of atherogenic small dense low-density lipoprotein particles leading to atherogenic plasma lipid profile and further increase the risk of cardiovascular disease[27]. There was mild variation in mean values of total cholesterol levels for cases of NALFD with diabetes (203.14 ± SD 43.11) (CI 197.05, 209.23) and non diabetic subjects (183.43 ± SD40.66) (CI 167.28, 187.81) (p=.0001). The current study demonstrates that total cholesterol and LDL levels were raised but not of much significance and well below the upper limit of reference range. Insulin also enhances cholesterol transport into arteriolar smooth muscle cells and increases endogenous lipid synthesis by these cells. Insulin also stimulates the proliferation of arteriolar smooth muscle cells, augments collagen synthesis in the vascular wall, increases the formation of and decreases the regression of lipid plaques, and stimulates the production of various growth factors which will lead to artherosclerosis[28]. Therefore, we conclude that non insulin dependent diabetes mellitus and dyslipidemia often coexist with NAFLD and the mortality due to cardiovascular risk possibly will compete with liver-related risk in dictating the final outcome.

Conclusion

Hepatic steatosis and non insulin dependent diabetes mellitus appears to be considerably allied. In addition, NALFD possibly represents an independent risk factor mainly due to atherogenic lipid profile which enhances the total cardiovascular risk further. Therefore, both hepatic steatosis and non insulin dependent diabetes mellitus

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exemplify a growing healthcare burden which will boost health care cost in the upcoming decades. Management of patients with NAFLD ought to be aimed at fighting the metabolic risk factors such as hyperglycemia and hypertriglyceridemia. Successful lifestyle adjustments with increased exercise and reduce food intake is able to get rid of excess fat in liver and can reverse insulin resistance.

Conflict of Interests

The authors do not have any conflict of interest arising from the study.

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