EJMA

DOI: 10.14744/ejma.2022.53825
EJMA 2022;2(3):118–125

Research Article

Determination of Chemotherapy Associated Fatty Liver Disease Among Colorectal Cancer Patients by Non-Invasive Methods

Ugur Sahin,[1] Musturay Karcaaltincaba,[2] Suayib Yalcin[3]

[1]Hematology and Stem Cell Transplantation Unit, Medicana International Ankara Hospital, Ankara, Türkiye
[2]Department of Radiology, Faculty of Medicine, Hacettepe University, Ankara, Türkiye
[3]Department of Clinical Oncology, Hacettepe University Institute of Oncology, Ankara, Türkiye

Abstract

Objectives: Chemotherapy-associated steatohepatitis (CASH) may increase the risk of cardiovascular, metabolic and liver-related complications. This study aimed to evaluate the frequency and severity of CASH and possible related factors during adjuvant chemotherapy for colorectal cancer (CRC) non-invasively.

Methods: Patients scheduled for adjuvant CRC treatment (FOLFOX-4 or FUFA) were assigned to prospective cohort (PC) or early (9-18 months) (ELT) and late (>18 months) long-term (LLT) follow-up and control groups (CG). PC was evaluated at baseline, third and sixth months for changes in anthropometric measurements, hemogram, blood biochemistry, inflammation markers, serum adipokine levels and hepatic steatosis by magnetic resonance spectroscopy (HS-MRS). The results of ELT, LLT and CG were compared.

Results: Of 21 patients included in PC, 66.7% received FOLFOX-4 and 33.3% FUFA. Males constituted 57.1%. Median age was 62.0 (48.0-77.0). Baseline characteristics were similar for both regimens. HS-MRS significantly increased at third and sixth months compared to baseline (p=0.02). ELT, LLT ve CG included 10, 10, and 20 patients, respectively. Age, gender distribution and anthropometric measurements were similar. HS-MRS tended to be higher in ELT (p=0.11).

Conclusion: Steatosis and CASH frequently develop during 5-fluorouracil and oxaliplatin treatment and may persist for months. Hepatic MRS and biomarkers may allow for non-invasive diagnosis.

Keywords: Chemotherapy, steatohepatitis, adipokine, magnetic resonance imaging

Cite This Article: *Sahin U, Karcaaltincaba M, Yalcin S. Determination of Chemotherapy Associated Fatty Liver Disease Among Colorectal Cancer Patients by Non-Invasive Methods. EJMA 2022;2(3):118–125.*

Chemotherapy-induced hepatotoxicity and/or exacerbation of pre-existing liver disease may lead to impaired liver function during treatment with cytotoxic drugs, targeted therapies, and immunotherapeutic drugs. Liver function tests should be closely monitored during treatment with these agents. Most hepatotoxic drug side effects, which are idiosyncratic, dose-related and unpredictable, occur via immunological or metabolic pathways.[1] The resulting cell damage is usually manifested by inflammation and/or cholestasis. Various different drugs are associated with specific histopathological changes in the liver as well. These changes are classified as non-alcoholic fatty liver disease (NAFLD) and vascular lesions. Their incidence varies according to the inciting agent, which includes anti-metabolites such as 5-FU and methotrexate, platinum derivatives, L-asparaginase, glucocorticoids, irinotecan, etc.[2-4] The presence of NAFLD increases the risk of metabolic comorbidities including cardiovascular disease, cerebrovascular events and diabetes mellitus (DM), and liver-related morbidity and mortality. Reversible improvement of these complications may take months after discontinuation of treatment.[5]

Address for correspondence: Ugur Sahin, MD. Hematoloji ve Kok Hucre Nakli Unitesi, Medicana International Ankara Hastanesi, Ankara, Türkiye
Phone: +90 312 292 92 92 **E-mail:** ugursahin_dr@yahoo.com

Submitted Date: August 11, 2022 **Accepted Date:** August 15, 2022 **Available Online Date:** October 10, 2022

©Copyright 2022 by Eurasian Journal of Medical Advances - Available online at www.ejmad.org
OPEN ACCESS This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

NAFLD refers to the changes in liver as a result of fat accumulation in hepatocytes and was first described as a histopathological entity in 1980.[6] It is regarded as the reflection of insulin resistance on liver and as part of the metabolic syndrome.[7] Its prevalence, which has been reported as 6-11% in autopsy series in the general population, is parallel to the epidemiology of obesity.[8, 9] Obesity, type 2 DM, hypothyroidism, metabolic syndrome, and many drugs and toxins can cause this condition.[10] In its mildest form, which is macrovesicular steatosis, fat accumulation in hepatocytes is histologically observed. The most severe form is non-alcoholic steatohepatitis (NASH), in which inflammation accompanies to fatty infiltration of the liver.[6] Progression to cirrhosis and hepatocellular carcinoma have been reported in cases with NASH.[11, 12] The first report of chemotherapy-associated steatohepatitis (CASH) was made in 1990 after the observation of hepatosteatosis development in areas of high drug perfusion in patients who received floxuridine infusion through hepatic artery.[13]

Chemotherapy-associated vascular lesions were first described in 2004 in tumor-free liver tissues of patients who underwent metastasectomy after neoadjuvant therapy for liver metastasis.[2] These lesions form severe sinusoidal enlargements that start in the centrilobular area and cause congestion and bleeding. In more severe cases, hemorrhagic centrilobular necrosis, fibrosis-related occlusions around the venules, and nodular regenerative hyperplasia are observed.[14] These lesions are generally referred to as "veno-occlusive disease (VOD)" or "sinusoidal obstruction syndrome (SOS)". VOD was first described in 1920 among patients with a fatal poisoning after ingestion of pyrrolizidine alkaloids found in some plants.[15] It is rarely seen except in the post-bone marrow transplantation period.[16, 17] Nodular regenerative hyperplasia, which is another specific vascular lesion type, may also lead to portal hypertension, splenomegaly, and cholestasis.[18]

Although liver biopsy is the golden standard in the evaluation of most hepatic lesions, there is a search for alternative non-invasive methods due to its difficulties. Conventional imaging methods and liver function tests cannot differentiate between simple steatosis and NASH. Liver magnetic resonance imaging (MRI) methods are promising in this regard. The combinations of various biochemical parameters including liver function tests, specific metabolic features, inflammatory markers and adipokine levels are being studied as indicators of hepatic damage.

This observational study aimed to determine the frequency and severity of CASH during adjuvant chemotherapy for colorectal cancer (CRC) by non-invasive methods including various clinical and biochemical parameters and radiological imaging, and to evaluate the and possible risk factors for the development of CASH.[19]

Methods

The candidate patients were assigned and evaluated in two main study groups including the prospective follow-up cohort (PC) and the long-term follow-up group. The results of patients in the long-term follow-up group were compared to a control group (CG) of newly diagnosed CRC patients. The long-term follow-up group was dichotomized according to the time elapsed from the start of treatment into early (9-18 months) (ELT) and late long-term follow-up groups (≥18 months) (LLT). Patients over 18 years of age and without gross residual hepatic tumor, who were admitted to the medical oncology unit between 01.10.2010 and 30.08.2011 to receive adjuvant treatment after curative surgery for CRC and who gave informed consent to participate in the study, were included in PC and CG. CRC patients over 18 years of age and without gross residual hepatic tumor, who had started and completed one of the investigated adjuvant chemotherapy regimens at least six months before the date of inclusion and who gave informed consent to participate in the study were included in ELT and LLT. Patients who received or planned to receive treatment with anti-VEGF and anti-EGFR agents (bevacizumab, cetuximab, etc.) were excluded. Patients included in PC were evaluated for investigated parameters at the time of enrollment and following third and sixth months. Patients in ELT, LLT and CG did not have follow-up visits after being evaluated for the investigated parameters at the time of study entry.

Investigated adjuvant treatments included FOLFOX-4 and FUFA regimens. FOLFOX-4 consisted of 85 mg/m^2 of oxaliplatin IV in 2 hours on day 1, folinic acid 200 mg/m^2 IV in 2 hours on days 1 and 2, 400 mg/m^2 of 5-fluorouracil (5-FU) IV bolus followed by 600 mg/m^2 22 hours IV infusion on days 1 and 2 every 2 weeks. This scheme was administered for a total of 12 times (6 cycles). Two different schemes were available for patients receiving FUFA. The majority of patients received FUFA as 425 mg/m^2 of 5-FU IV and 20 mg/m^2 of folinic acid IV on days 1 to 5 every 28 days. This scheme was was administered for a total of 6 times (6 cycles). Few patients received FUFA as 425 mg/m^2 of 5-FU IV and 20 mg/m^2 of folinic acid IV once a week for a total of 24 weeks (6 cycles). In the third month follow-up, 78.5% of patients receiving FOLFOX-4 and 85.7% of patients receiving FUFA completed their 3-cycle treatment. At the sixth month follow-up, 92.9% of patients receiving FOLFOX-4 and 85.7% of patients receiving FUFA completed their 6-cycle treatment. All patients in ELT and LLT had received 6 cycles of treatment.

Height, body weight, waist and hip circumference measurements were made by the same investigator at baseline, third and sixth month follow-up visits. Standard instruments were used for height and body weight measurements. Height and weight measurements were made while looking forward in an upright position without shoes and while clothed. Body mass index (BMI) was calculated ac-

cording to the formula.[20] Waist and hip circumferences were measured according to the NHANES (National Health and Nutrition Examination Survey) III protocol.[20] A waist circumference of >102 cm in men and >88 cm in women, a waist to hip circumference ratio of >0.9 in men and >0.85 in women were accepted as an indicator of visceral obesity.[21]

Vertical liver and spleen size and liver fat percentage were determined by hepatic 1H-magnetic resonance spectroscopy (MRS) at baseline, third and sixth month follow-up visits. MRI device with 1.5 Tesla was used in MRS scans. In the localization images, a 20x20x20mm voxel was placed in a region free of major vessels and bile ducts (in segment 5-6 of the right lobe). MRS images were obtained without water suppression by using the stimulated echo acquisition mode (STEAM) method and the hepatic fat fraction was measured from the spectroscopic image. For the STEAM method, TR/TE: 2500 ms/10 ms, BW: 2500 Hz, average: 2, readout points: 2048, shooting time: 20 sec were used and the shots were taken while holding a single breath. A calculated lipid ratio of ≥5.0% was accepted as the cut-off for significant hepatic steatosis. A vertical liver/spleen size of ≥12.0 cm was accepted as the presence of hepato/splenomegaly. All measurements and calculations were performed blindly by the same radiologist.

Venous blood samples were obtained after at least eight hours of fasting at baseline, third and sixth month follow-up visits. Analyses of fasting blood glucose, serum total cholesterol, LDL (low density lipoprotein), HDL (high density lipoprotein), triglycerides, AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), GGT (gamma glutamyl transpeptidase), total bilirubin, albumin, homocysteine, calcium, magnesium, uric acid, ferritin, CRP (C reactive protein), complete blood count, HBsAg, anti-HBs, anti-HCV were performed per general standards of practice immediately after collection of appropriate blood samples. For the analyses of serum leptin, adiponectin, resistin and plasma ghrelin levels samples obtained by centrifugation of blood samples at +4ºC at 4000 rpm for 10 minutes were stored at -30ºC until the analysis. Serum adipokine levels were studied with commercially available ELISA or EIA kits and in accordance with the protocols recommended by the manufacturers. Serum samples were used for leptin, adiponectin and resistin tests, and plasma samples were used for ghrelin. Leptin (sandwich) enzyme-linked immunosorbent assay (ELISA) (DRG Instruments GmbH®, Germany), total human adiponectin ELISA (TECO Medical Group®, Switzerland), human resistin platinum ELISA (Bender MedSystems GmbH®, Austria) and human ghrelin enzyme immunoassay (EIA) (Phoenix Europe GmbH®, Germany) commercial kits were used.

The study was approved by the institutional non-Invasive clinical research ethics committee (decision number and date: HEK11/18-28, February 3, 2011). The study was conducted in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments. Verbal and written informed consents were obtained from all patients before inclusion in the study.

Descriptive statistics of categorical variables were given as numbers and percentages. Normal distribution of numerical variables was evaluated by visual (histograms and probability graphs) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). None of the investigated numerical variables were normally distributed. Descriptive statistics for all numerical variables were calculated as the median (minimum and maximum values). For nominal variables of dependent samples, Cochran's Q test was used when comparing more than two variables, and McNemar test was used for pairwise comparisons. For ordinal and numerical variables, Friedman test was used when more than two variables were compared, and the Wilcoxon test was used for pairwise comparisons. Chi-square or, where appropriate, Fisher's test were used for nominal variables of independent samples. Kruskal-Wallis test was used when comparing more than two variables for ordinal and numerical variables, and the Mann-Whitney U test was used for pairwise comparisons. A type 1 error of <5% was considered statistically significant in all analyses. Statistical package program SPSS® Statistics 25.0 (IBM Corp. released 2017. Armonk, NY, USA) was used for all calculations.

Findings

PC included 21 patients with CRC in stage 2a (28.6%, n=6), stage 2b (14.3%, n=3), stage 3b (42.9%, n=9) and stage 3c (9.5%, n=3). Stage 4 disease was present only in one patient, who had exclusively liver metastases and undergone total metastasectomy before adjuvant chemotherapy. Neoadjuvant chemoradiotherapy with 5-FU was administered to two patients (9.5%) with stage 2a and 3b rectal cancer. Of the patients, 14 (66.7%) were treated with FOLFOX-4 and 7 (33.3%) with FUFA regimen. Males constituted 57.1% (n=12). Median age was 62.0 (48.0-77.0) (Table 1). Median

Table 1. Baseline characteristics of patients in the prospective follow-up cohort

Gender, n (%)	
Male	12 (57.1)
Female	9 (42.9)
Age, median (minimum-maximum)	62 (48-77)
Metabolic syndrome, n (%)	12 (57.1)
Type 2 diabetes mellitus, n (%)	4 (19.0)
Coronary artery disease, n (%)	4 (19.0)
Smoking, n (%)	6 (28.6)
Alcohol, n (%)	4 (19.0)
Chronic viral hepatitis, n (%)	2 (9.5)
Chemotherapy regimen, n (%)	
FOLFOX-4	14 (66.7)
FUFA	7 (33.3)

FOLFOX-4: folinic acid, 5-fluorouracil and oxaliplatin; FUFA: 5-fluorouracil and folinic acid.

body weight and BMI at baseline was 73.0 kg (60.0-89.0) and 27.0 kg/m^2 (22.3-35.4), respectively (Table 2). Obese or overweight patients constituted 66.7%. The distribution of baseline characteristics was similar among patients receiving FOLFOX-4 and FUFA. Metabolic syndrome was present in 57.1% (n=12), type 2 DM in 19.1% (n=4), hypertension in 23.8% (n=5), coronary artery disease (CAD) in 14.3% (n=3) and hyperlipidemia in 28.6% (n=6) of the patients (Table 1). History of smoking and regular alcohol intake were present in 38.1% (n=8) and 14.3% (n=3), respectively (Table 1). Two patients (9.5%) were serologically positive for hepatitis C virus (HCV).

Anthropometric measurements did not significantly change between the follow-up visits of patients in PC (Table 2). Hepatic steatosis measurement by MRS (HS-MRS) increased significantly at the third and sixth month visits compared to the baseline (p=0.02). Increases in serum HDL, AST, total and direct bilirubin levels when compared to baseline were statitistically significantly (p=0.002, p=0.01, p=0.01 and p=0.007, respectively) (Table 2). Significant decreases were observed in serum homocysteine and platelet levels at the third and sixth month visits (p=0.008 and p=0.001, respectively). Serum adiponectin and resistin levels decreased at third month visit when compared to

Table 2. Evaluation of changes observed in studied parameters of patients in the prospective follow-up cohort

Parameters	Baseline median (minimum-maximum)	3rd month median (minimum-maximum)	6th month median (minimum-maximum)	p
BMI, kg/m^2	27.0 (22.3 – 35.4)	26.6 (21.2 – 36.2)	26.7 (22.3 – 38.5)	0.93
Waist circumference, cm	95.0 (87.0 – 115.0)	94.0 (84.0 – 114.0)	93.0 (84.0 – 111.0)	0.20
Hip circumference, cm	96.5 (85.0 – 119.0)	97.5 (85.0 – 118.0)	99.0 (82.0 – 124.0)	0.27
Waist/hip ratio	0.97 (0.88 – 1.09)	1.01 (0.85 – 1.12)	0.97 (0.80 – 1.15)	0.58
Fasting blood glucose, mg/dL	98.0 (79.0 – 169.0)	116.0 (85.0 – 309.0)	108.0 (71.0 – 306.0)	0.29
Serum total cholesterol, mg/dL	203.0 (127.0 – 385.0)	196.0 (133.0 – 247.0)	199.0 (170.0 – 305.0)	0.88
Serum LDL, mg/dL	124.0 (63.0 – 315.0)	114.0 (32.0 – 170.0)	124.0 (63.0 – 207.0)	0.26
Serum HDL, mg/dL	46.0 (26.0 – 74.0)	47.0 (24.0 – 86.0)	56.0 (41.0 – 93.0)	0.002
Serum triglycerides, mg/dL	136.5 (64.0 – 515.0)	159.5 (57.0 – 824.0)	160.0 (79.0 – 490.0)	0.91
Serum AST, U/L	17.0 (8.0 – 39.0)	27.0 (11.0 – 56.0)	25.5 (11.0 – 79.0)	0.01
Serum ALT, U/L	15.0 (7.0 – 46.0)	17.0 (7.0 – 64.0)	17.5 (9.0 – 55.0)	0.17
Serum ALP, U/L	83.0 (61.0 – 122.0)	96.0 (62.0 – 148.0)	104.5 (78.0 – 161.0)	0.07
Serum GGT, U/L	31.0 (11.0 – 139.0)	34.0 (10.0 – 103.0)	29.5 (17.0 – 97.0)	0.65
Serum total bilirubin, mg/dL	0.36 (0.22 – 0.99)	0.46 (0.16 – 1.82)	0.44 (0.28 – 1.56)	0.01
Serum direct bilirubin, mg/dL	0.12 (0.01 – 0.36)	0.18 (0.09 – 0.90)	0.16 (0.11 – 0.61)	0.007
Serum albumin, g/dL	4.48 (2.89 – 5.01)	4.28 (3.50 – 4.57)	4.21 (3.63 – 4.85)	0.20
Serum total calcium, mg/dL	9.90 (8.85 – 10.57)	9.56 (8.81 – 10.18)	9.55 (8.98 – 10.32)	0.65
Serum magnesium, mg/dL	2.0 (1.5 – 2.3)	2.0 (1.1 – 2.3)	1.95 (1.31 – 2.26)	0.26
Serum uric acid, mg/dL	5.0 (2.9 – 8.5)	4.6 (3.6 – 8.4)	4.8 (3.4 – 7.6)	0.95
Serum CRP, mg/dL	0.68 (0.16 – 9.84)	0.40 (0.20 – 10.7)	0.34 (0.33 – 2.55)	0.59
Serum homocysteine, µmol/L	16.57 (12.21 – 22.38)	12.57 (9.18 – 20.24)	14.17 (8.66 – 22.08)	0.008
Serum ferritin, ng/mL	53.5 (9.4 – 569.0)	81.1 (8.7 – 735.0)	87.1 (19.4 – 367.7)	0.10
Serum leptin, ng/mL	4.89 (0.79 – 30.87)	3.42 (0.71 – 44.13)	5.54 (1.71 – 100.00)	0.56
Serum adiponectin, µg/mL	15.22 (2.29 – 76.16)	10.87 (2.23 – 90.89)	13.27 (5.97 – 100.00)	0.04
Serum resistin, ng/mL	156.57 (52.76 – 1047.50)	124.15 (33.24 – 668.38)	203.29 (55.96 – 672.68)	0.006
Serum ghrelin, ng/mL	8.88 (5.67 – 73.24)	9.55 (8.12 – 65.95)	9.61 (7.60 – 27.03)	0.11
Hemoglobin, g/dL	12.8 (9.4 – 15.1)	11.5 (9.8 – 14.2)	12.3 (10.1 – 14.6)	0.31
Leukocyte count, 10^9/L	6.9 (4.0 – 12.7)	4.3 (2.2 – 14.0)	4.95 (2.4 – 16.7)	0.13
Platelet count, 10^9/L	337.0 (178.0 – 645.0)	195.0 (43.0 – 304.0)	180.0 (77.0 – 295.0)	0.001
Vertical liver size, cm	17.0 (13.0 – 21.0)	16.7 (11.0 – 21.0)	16.7 (11.8 – 19.5)	0.43
Vertical spleen size, cm	11.0 (8.6 – 13.3)	12.0 (8.0 – 13.9)	12.0 (9.3 – 14.5)	0.10
Hepatic steatosis by MRS, %	3.0 (2.0 – 7.0)	4.0 (2.0 – 15.0)	5.3 (1.0 – 17.0)	0.02

BMI: body mass index; LDL: low density lipoprotein; HDL: high density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma glutamyl transpeptidase; CRP: C reactive protein; MRS: magnetic resonance spectroscopy.

baseline and increased again at sixth month visit (p=0.04 and p=0.006, respectively). Changes observed in other parameters were not significant (Table 2).

The frequency of having a serum AST level of >25 U/L at third and sixth month visits, and an ALP level of >100 U/L at sixth month visit were significantly higher among PC patients who were scheduled for FOLFOX-4 regimen than FUFA (p=0.01, p=0,04 and p=0,04, respectively). Among PC patients receiving FOLFOX-4 regimen there was also an observed tendency for having an ALP level of >100 U/L and a homocysteine level of ≤15 µmol/L at third month, and a GGT level of >40 U/L and a vertical spleen size of ≥12 cm at sixth month visits when compared to FUFA (p=0.11, p=0.09 p=0.12 and p=0.07, respectively) (Table 3).

CG, ELT and LLT included 20, 10 and 10 patients, respectively. Of the 20 patients in CG, 30.0% (n=6) had stage 2a, 20.0% (n=4) stage 2b, 35.0% (n=7) stage 3b, 5.0% (n=1) stage 3c and 10.0% (n=2) stage 4 CRC. Of the patients who completed FOLFOX-4, 14.3% (n=2) had stage 2a, 28.6% (n=4) stage 2b, 35.7% (n=5) stage 3b, and 21.4% (n=3) stage 3c CRC. Of the patients who completed FUFA 33.3% (n=2) had stage 2a, 50.0% (n=3) stage 2b, and 16.7% (n=1) stage 3b CRC. There was no difference between CG, ELT and LLT groups in terms of distribution of age, gender and anthropometric measurements. Median HS-MRS in ELT was higher than CG and LLT, but the difference was not significant (p=0.11). Median serum AST, ALP and albumin levels were significantly higher in ELT when compared to CG and LLT (p=0.008, p=0.04 and p=0.02, respectively). Median serum homocysteine and adiponectin levels were significantly lower in ELT when compared to CG and LLT (p=0.04 and p=0.02, respectively). Median platelet count was significantly lower in ELT and LLT groups than in CG (p=0.01). Median serum GGT was significantly lower in LLT (p=0.009) (Table 4).

Discussion

This prospective observational study involved patients with CRC who received FOLFOX-4 or FUFA as adjuvant therapy and investigated various biochemical and clinical parameters that may be associated with the development of radiological fatty liver disease and chemotherapy-induced hepatotoxicity. The study population was considered to be at risk for the development of hepatotoxicity since the baseline frequencies of metabolic syndrome and obesity was very high. The observations can be directly interpreted as the effects of chemotherapy on the liver, as there were no significant changes in BMI and other anthropometric measurements during the follow-up.

HS-MRS increases during the chemotherapy period. This condition may persist until 18 months after the start of chemotherapy, and may regress approximately over 18 months. The reported acute steatosis rates of approximately 50.0% in patients receiving infusional 5-FU and oxaliplatin are consistent with our findings (steatosis rates in the third and sixth month visits were 30% and 54.5% for FOLFOX-4 and 42.9% and 40.0% for FUFA).[22] However, it is not clear whether similar results would be observed in a group of normal weight or underweight individuals, since the majority of patients included in our study had a high baseline frequency of metabolic syndrome, overweight or obesity.

The observed changes in serum AST, ALT, total and direct bilirubin, homocysteine and platelet levels during the chemotherapy period are statistically significant. However, they hardly possess clinical significance since these changes are mainly within reference values. The course of these changes also differ among patients treated with FOLFOX-4 and FUFA. This may be related to the fact that different agents cause different histopathological conditions in the

Table 3. The frequency of changes in selected parameters according to chemotherapy regimens in the prospective follow-up cohort

	Baseline		P	3rd month		P	6th month		P
	FOLFOX-4	FUFA		FOLFOX-4	FUFA		FOLFOX-4	FUFA	
AST >25 U/L	14.3	28.6	0.43	71.4	14.3	0.01	72.7	20.0	0.04
ALT >25 U/L	21.4	28.6	0.72	35.7	14.3	0.31	45.5	20.0	0.33
ALP >100 U/L	21.4	28.6	0.72	50.0	14.3	0.11	72.7	20.0	0.04
GGT >40 U/L	35.7	28.6	0.74	28.6	14.3	0.47	36.4	-	0.12
Thrombocytopenia (<150 x10^3/µL)	-	-	-	35.7	14.3	0.31	36.4	20.0	0.51
Homocysteine >15 µmol/L	81.8	40.0	0.09	18.2	57.1	0.09	30.0	40.0	0.70
Vertical liver size ≥12 cm	100.0	100.0	-	100.0	85.7	0.20	81.8	100.0	0.93
Vertical spleen size ≥12 cm	44.4	16.7	0.26	60.0	28.6	0.49	70.0	20.0	0.07
Hepatic steatosis by MRS ≥5.0%	20.0	16.7	0.87	30.0	42.9	0.59	54.5	40.0	0.59

FOLFOX-4: folinic acid, 5-fluorouracil and oxaliplatin; FUFA: 5-fluorouracil and folinic acid; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma glutamyl transpeptidase; MRS: magnetic resonance spectroscopy.

Table 4. Comparison of patients in the early and long term follow-up and control groups

Parameters	Control	Early (9-18 months)	Late (≥18 months)	P
Gender, n (%)				
Male	10 (50.0)	6 (66.7)	6 (54.5)	0.71
Female	10 (50.0)	3 (33.3)	5 (45.5)	
Age, median (minimum-maximum)	64 (48 – 77)	60 (38 – 70)	60 (44 – 78)	0.54
Metabolic syndrome, n (%)	13 (65.0)	5 (55.6)	5 (45.5)	0.57
Type 2 diabetes mellitus, n (%)	4 (20.0)	2 (22.2)	–	0.26
Coronary artery disease, n (%)	4 (20.0)	1 (11.1)	1 (9.1)	0.67
Smoking, n (%)	5 (25.0)	6 (66.7)	5 (45.5)	0.10
Alcohol, n (%)	3 (15.0)	4 (44.4)	–	0.03
Chronic viral hepatitis, n (%)	2 (10.0)	–	–	0.35
Chemoterapy regimen, n (%)				
FOLFOX-4	–	8 (88.9)	6 (54.5)	0.10
FUFA	–	1 (11.1)	5 (45.5)	
BMI, kg/m^2, median (minimum-maximum)	27.4 (22.3 – 35.4)	29.9 (19.3 – 35.3)	28.5 (21.3 – 46.5)	0.53
Waist circumference, cm, median (minimum-maximum)	95.0 (77.0 – 115.0)	97.0 (74.0 – 118.0)	89.0 (76.0 – 123.0)	0.52
Hip circumference, cm, median (minimum-maximum)	96.5 (91.0 – 119.0)	100.0 (93.0 – 112.0)	98.0 (87.0 – 139.0)	0.60
Waist/hip, median (minimum-maximum)	0.96 (0.84 – 1.09)	0.98 (0.78 – 1.05)	0.89 (0.79 – 1.05)	0.14
Fasting blood glucose, mg/dL, median (minimum-maximum)	99.5 (79.0 – 169.0)	102.0 (89.0 – 169.0)	96.0 (74.0 – 126.0)	0.62
Serum total cholesterol, mg/dL, median (minimum-maximum)	209.0 (127.0 – 385.0)	211.0 (175.0 – 241.0)	224.0 (175.0256.0)	0.93
Serum LDL, mg/dL, median (minimum-maximum)	127.0 (63.0 – 315.0)	132.0 (95.0 – 160.0)	135.0 (101.0169.0)	0.99
Serum HDL, mg/dL, median (minimum-maximum)	47.0 (26.0 – 74.0)	49.0 (26.0 – 65.0)	49.0 (37.0 – 180.0)	0.40
Serum triglycerides, mg/dL, median (minimum-maximum)	132.0 (64.0 – 515.0)	222.0 (110.0 – 472.0)	143.0 (64.0 – 235.0)	0.14
Serum AST, U/L, median (minimum-maximum)	17.00 (8.0 – 39.0)	28.0 (19.0 – 34.0)	19.0 (15.0 – 24.0)	0.008
Serum ALT, U/L, median (minimum-maximum)	15.0 (7.0 – 46.0)	21.0 (17.0 – 32.0)	15.0 (10.0 – 28.0)	0.09
Serum ALP, U/L, median (minimum-maximum)	82.0 (61.0 – 122.0)	115.0 (66.0 – 183.0)	84.0 (57.0 – 130.0)	0.04
Serum GGT, U/L, median (minimum-maximum)	32.0 (11.0 – 139.0)	31.0 (25.0 – 221.0)	15.0 (6.0 – 42.0)	0.009
Serum total bilirubin, mg/dL, median (minimum-maximum)	0.34 (0.22 – 0.99)	0.55 (0.32 – 1.17)	0.35 (0.20 – 1.05)	0.11
Serum direct bilirubin, mg/dL, median (minimum-maximum)	0.11 (0.01 – 0.36)	0.19 (0.09 – 0.30)	0.12 (0.07 – 0.29)	0.15
Serum albumin, g/dL median (minimum-maximum)	4.49 (2.89 – 5.01)	4.77 (4.36 – 5.43)	4.65 (3.67 – 5.17)	0.02
Serum total calcium, mg/dL median (minimum-maximum)	9.94 (8.85 – 10.57)	9.95 (9.49 – 10.30)	9.71 (8.64 – 10.37)	0.73
Serum magnesium, mg/dL, median (minimum-maximum)	2.1 (1.5 – 2.3)	2.1 (1.9 – 2.2)	2.0 (1.9 – 2.1)	0.71
Serum uric acid, mg/dL, median (minimum-maximum)	4.9 (2.9 – 6.3)	6.2 (3.1 – 8.7)	4.8 (4.1 – 7.7)	0.17
Serum CRP, mg/dL, median (minimum-maximum)	0.59 (0.16 – 9.84)	0.39 (0.24 – 1.09)	0.36 (0.13 – 0.98)	0.12
Serum homocysteine, µmol/L, median (minimum-maximum)	16.41 (12.21 – 22.38)	11.83 (9.19 – 17.78)	17.90 (10.33 – 27.14)	0.04
Serum ferritin, ng/mL, median (minimum-maximum)	58.8 (9.4 – 569.0)	17.4 (8.0 – 224.6)	44.2 (5.6 – 217.0)	0.28
Serum leptin, ng/mL, median (minimum-maximum)	6.02 (0.79 – 30.87)	9.12 (1.21 – 28.57)	13.45 (0.50 – 53.78)	0.52
Serum adiponectin, µg/mL, median (minimum-maximum)	15.50 (2.29 – 76.16)	6.65 (3.29 – 15.87)	16.70 (6.77 – 38.86)	0.02
Serum resistin, ng/mL, median (minimum-maximum)	169.98 (52.76 – 1047.50)	178.63 (84.53 – 414.66)	253.83 (103.47 – 688.14)	0.58
Serum ghrelin, ng/mL, median (minimum-maximum)	8.93 (5.67 – 73.24)	9.29 (7.49 – 24.60)	24.60 (8.36 – 53.78)	0.09
Hemoglobin, g/dL, median (minimum-maximum)	12.9 (9.4 – 15.1)	14.1 (11.4 – 18.3)	13.40 (9.0 – 15.6)	0.16
Leukocyte count, 10^9/L, median (minimum-maximum)	6.7 (4.0 – 10.6)	7.6 (4.0 – 9.6)	6.4 (4.9 – 9.4)	0.43
Platelet count, 10^9/L, median (minimum-maximum)	340.5 (178.0 – 645.0)	253.0 (100.0 – 276.0)	254.0 (184.0 – 364.0)	0.01
Vertical liver size, cm, median (minimum-maximum)	17.0 (13.0 – 21.0)	16.5 (15.0 – 18.3)	16.3 (15.2 – 20.1)	0.82
Vertical spleen size, cm, median (minimum-maximum)	11.0 (8.6 – 13.3)	12.0 (10.0 – 13.5)	10.7 (9.5 – 12.0)	0.38
Hepatic steatosis by MRS, % (minimum-maximum)	3.0 (2.0 – 7.0)	7.0 (2.0 – 20.0)	3.5 (2.5 – 8.7)	0.11

FOLFOX-4: folinic acid, 5-fluorouracil and oxaliplatin; FUFA: 5-fluorouracil and folinic acid; BMI: body mass index; LDL: low density lipoprotein; HDL: high density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma glutamyl transpeptidase; CRP: C reactive protein; MRS: magnetic resonance spectroscopy.

liver (steatosis, CASH, STS, etc.). Oxaliplatin has been associated predominantly with vascular damage in the liver, leading to the development of STS.[2] On the other hand, 5-FU causes acute steatosis, which is mostly reversibly after administration.[22] Although these changes in biochemical parameters may not be very useful in clinical practice alone, they can be used as a parameter of artificial intelligence algorithms designed for the evaluation of CASH risk in further studies.

The sinusoidal damage caused by oxaliplatin is associated with the development of splenomegaly.[23] Our observation of respective splenomegaly frequencies of 70.0% and 20.0% in FOLOFOX-4 and FUFA groups in the sixth month visit may support this finding. The persistance of splenomegaly in patients who received FOLFOX-4 may possibly demonstrate that the clinical impact becomes more pronounced months after the end of treatment. Subclinical portal hypertension due to oxaliplatin as a result of steatohepatitis and fibrotic changes in the liver in the late period may be responsible for splenomegaly.

Leptin has been reported to exert protective effects against steatosis. However, it is profibrogenic, and serum leptin levels are proportional to body fat mass.[24] Serum levels may correlate with the severity of steatosis, but its relationship with fibrosis is not clear.[25] We did not observe a significant change in the leptin levels of patients who received FOLFOX-4 and FUFA. A possible explanation may be the masking of possible chemotherapy related effects by the high prevalence of overweight or obesity, since BMI is the main determinant of serum leptin levels.

Adiponectin reduces inflammation by increasing the release of anti-inflammatory cytokines. Serum levels are inversely proportional to body fat mass and decrease in obesity and type 2 DM.[26] No significant changes were observed in adiponectin levels in the follow-up of PC patients. However, our observation of lower adiponectin levels in ELT group compared to LLT and CG may indicate the continuation of inflammatory response in this period.

Resistin is involved in the development of inflammation and fibrosis in humans.[26] Ghrelin levels are reported to negatively correlate with BMI and increased levels may cause hepatic steatosis.[27, 28] The role of resistin and ghrelin in the pathogenesis of CASH may be more limited since we did not observe any difference in serum levels among patients who received FOLFOX-4 and FU-FA.

Liver biopsy is still the only golden standard for the diagnosis of hepatic steatosis, steatohepatitis, hepatic vascular lesions and fibrosis.[29] However, the progress in non-invasive methods such as functional MRI techniques is promising. Among these, MRS is reported to be the most precise method to evaluate the amount of hepatic steatosis [30]. The ratio of the amount of triglycerides to the amount of water contained in all hepatocytes is roughly calculated with 1H-MRS. Correlation with the presence of steatosis in histopathological examination has been reported when the percentage of adiposity measured by MRS is ≥5.0%. Therefore, the threshold value for steatosis is accepted as ≥5.0%.[30] It should not be forgotten that the results obtained with MRS are at molecular level and pathological features such as the presence of inflammation, hepatocyte ballooning, and fibrosis that cannot be evaluated with MRS alone. Therefore, the presence of steatohepatitis and cirrhosis cannot be diagnosed solely by MRS.

The inability to control other possible effects on the results (course of comorbid conditions, concomitant drug use, special diet practices) and the heterogeneity of the patients included in the study make it difficult to interpret the results obtained from this study. On the contrary, the evaluation of various parameters in this patient group and the prospective design are the strengths of the study.

In conclusion, the development of hepatic steatosis is frequently observed during treatment with 5-FU and oxaliplatin. Liver MRS is an important non-invasive diagnostic tool for the diagnosis of hepatic steatosis. Further studies are needed to evaluate the role of possible biomarkers in predicting the development and severity of CASH. Besides the routine evaluation of liver functions during chemotherapy period, it should be kept in mind that that the nagative impacts may persist months after the end of chemotherapy. The education and guidance of patients during the treatment course in terms of correctable risk factors such as obesity, physical inactivity and metabolic syndrome may provide clinical benefit.

Disclosures

Ethics Committee Approval: Hacettepe University Scientific Research Ethical Committee. Date: February 3, 2011. Issue: HEK 11/18-28.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – S.Y., M.K.; Design – S.Y., M.K., U.S.; Supervision – S.Y., M.K.; Materials – U.S., S.Y., M.K.; Data collection and/or processing – U.S., S.Y., M.K.; Analysis and/or interpretation – U.S., S.Y., M.K.; Literature search – U.S., S.Y., M.K.; Writing – U.S., S.Y., M.K.; Critical review – S.Y., M.K.

References

1. Lee WM. Drug-induced hepatotoxicity. N Engl J Med 1995;333:1118–27.
2. Rubbia-Brandt L, Audard V, Sartoretti P, Roth AD, Brezault C, Le Charpentier M, et al. Severe hepatic sinusoidal obstruction

associated with oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. Ann Oncol 2004;15:460–6.
3. Vauthey JN, Pawlik TM, Ribero D, Wu TT, Zorzi D, Hoff PM, et al. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. J Clin Oncol 2006;24:2065–72. [CrossRef]
4. Ben-Yakov G, Alao H, Haydek JP, Fryzek N, Cho MH, Hemmati M, et al. Development of hepatic steatosis after chemotherapy for non-Hodgkin lymphoma. Hepatol Commun 2018;3:220–6.
5. Meunier L, Larrey D. Chemotherapy-associated steatohepatitis. Ann Hepatol 2020;19:597–601. [CrossRef]
6. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980;55:434–8.
7. Hilden M, Christoffersen P, Juhl E, Dalgaard JB. Liver histology in a 'normal' population--examinations of 503 consecutive fatal traffic casualties. Scand J Gastroenterol 1977;12:593–7.
8. McCullough AJ. Update on nonalcoholic fatty liver disease. J Clin Gastroenterol 2002;34:255–62. [CrossRef]
9. Mulhall BP, Ong JP, Younossi ZM. Non-alcoholic fatty liver disease: an overview. J Gastroenterol Hepatol 2002;17:1136–43.
10. Teli MR, James OF, Burt AD, Bennett MK, Day CP. The natural history of nonalcoholic fatty liver: a follow-up study. Hepatology 1995;22:1714–9. [CrossRef]
11. McCullough AJ. Pathophysiology of nonalcoholic steatohepatitis. J Clin Gastroenterol 2006;40:S17–29.
12. Regimbeau JM, Colombat M, Mognol P, Durand F, Abdalla E, Degott C, et al. Obesity and diabetes as a risk factor for hepatocellular carcinoma. Liver Transpl 2004;10:S69–73. [CrossRef]
13. Zeiss J, Merrick HW, Savolaine ER, Woldenberg LS, Kim K, Schlembach PJ. Fatty liver change as a result of hepatic artery infusion chemotherapy. Am J Clin Oncol 1990;13:156–60.
14. Aloia T, Sebagh M, Plasse M, Karam V, Lévi F, Giacchetti S, et al. Liver histology and surgical outcomes after preoperative chemotherapy with fluorouracil plus oxaliplatin in colorectal cancer liver metastases. J Clin Oncol 2006;24:4983–90.
15. Willmot FC, Robertson GW. Senecio disease, or cirrhosis of the liver due to senecio poisoning. Lancet 1920;196:848–9.
16. El-Sayed MH, El-Haddad A, Fahmy OA, Salama II, Mahmoud HK. Liver disease is a major cause of mortality following allogeneic bone-marrow transplantation. Eur J Gastroenterol Hepatol 2004;16:1347–54. [CrossRef]
17. Reiss U, Cowan M, McMillan A, Horn B. Hepatic venoocclusive disease in blood and bone marrow transplantation in children and young adults: incidence, risk factors, and outcome in a cohort of 241 patients. J Pediatr Hematol Oncol 2002;24:746–50.
18. Wanless IR. Micronodular transformation (nodular regenerative hyperplasia) of the liver: a report of 64 cases among 2,500 autopsies and a new classification of benign hepatocellular nodules. Hepatology 1990;11:787–97. [CrossRef]
19. Şahin U, Karçaaltıncaba M, Yalçın Ş. Kolorektal kanserli olgularda kemoterapi ilişkili steatohepatitin invazif olmayan yöntemlerle değerlendirilmesi. Tıpta Uzmanlık Tezi. Hacettepe Üniversitesi; 2012.
20. National Institutes of Health. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults--the evidence report. Obes Res 1998;6:51S–209S.
21. Ness-Abramof R, Apovian CM. Waist circumference measurement in clinical practice. Nutr Clin Pract 2008;23:397–404.
22. Jiménez R, Hijona E, Emparanza J, Alústiza JM, Hijona L, Macarulla MT, et al. Effect of neoadjuvant chemotherapy in hepatic steatosis. Chemotherapy 2012;58:89–94. [CrossRef]
23. Overman MJ, Maru DM, Charnsangavej C, Loyer EM, Wang H, Pathak P, et al. Oxaliplatin-mediated increase in spleen size as a biomarker for the development of hepatic sinusoidal injury. J Clin Oncol 2010;28:2549–55. [CrossRef]
24. Rabe K, Lehrke M, Parhofer KG, Broedl UC. Adipokines and insulin resistance. Mol Med 2008;14:741–51.
25. Chitturi S, Farrell G, Frost L, Kriketos A, Lin R, Fung C, et al. Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? Hepatology 2002;36:403–9. [CrossRef]
26. Tsochatzis EA, Papatheodoridis GV, Archimandritis AJ. Adipokines in nonalcoholic steatohepatitis: from pathogenesis to implications in diagnosis and therapy. Mediators Inflamm 2009;2009:831670. [CrossRef]
27. Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, et al. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. Biochem Biophys Res Commun 2003;300:472–6.
28. Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, et al. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab 2002;87:240–4. [CrossRef]
29. El-Badry AM, Breitenstein S, Jochum W, Washington K, Paradis V, Rubbia-Brandt L, et al. Assessment of hepatic steatosis by expert pathologists: the end of a gold standard. Ann Surg 2009;250:691–7.
30. Friedrich-Rust M, Müller C, Winckler A, Kriener S, Herrmann E, Holtmeier J, et al. Assessment of liver fibrosis and steatosis in PBC with FibroScan, MRI, MR-spectroscopy, and serum markers. J Clin Gastroenterol 2010;44:58–65. [CrossRef]