CANCER GENOMICS & PROTEOMICS *16*: 519-530 (2019)
doi:10.21873/cgp.20154

Synergism of Adipocytokine Profile and *ADIPOQ*/*TNF-α* Polymorphisms in NAFLD-associated MetS Predict Colorectal Liver Metastases Outgrowth

ROSA DIVELLA[1], ANTONELLA DANIELE[1], RAFFAELE DE LUCA[2], ANTONIO MAZZOCCA[3], EUSTACHIO RUGGIERI[2], EUFEMIA SAVINO[4], PORZIA CASAMASSIMA[4], MICHELE SIMONE[2], CARLO SABBA[3] and ANGELO PARADISO[1]

[1]*Institutional BioBank, Experimental Oncology and Biobank Management Unit, IRCCS Istituto Tumori "Giovanni Paolo II", Bari, Italy;*
[2]*Department of Surgery Oncology, IRCCS Istituto Tumori "Giovanni Paolo II", Bari, Italy;*
[3]*Interdisciplinary Department of Medicine, University of Bari School of Medicine, Bari, Italy;*
[4]*Clinical Pathology Laboratory, IRCCS Istituto Tumori "Giovanni Paolo II", Bari, Italy*

Abstract. *Background/Aim: The aim of this study was to evaluate whether the altered profile of adipocytokine and genetic fingerprint in NAFLD-associated metabolic syndrome "cluster" represents synergistic risk factors predicting onset of liver colorectal cancer metastases. Materials and Methods: A total of 165 colorectal cancer patients were enrolled, 56,3% were with metabolic syndrome/NAFLD. Serum samples were assayed for ADIPOQ, leptin and TNF-a levels by ELISA. ADIPOQ rs266729 C/G and TNF-308 A/G genotypes were analyzed in DNA isolated from whole blood. Results: Reduction in adiponectin levels and increase in leptin and TNF-α was shown in patients with liver metastases. This trend was influenced by BMI, MetS/NAFLD, and insulin resistance. ADIPOQ G rs266729 and TNF- 308 A allele are associated with obesity, MetS/NAFLD and insulin resistance. ADIPOQ CG/GG and GA/AA TNF-alpha genotypes confer susceptibility to liver metastases. Conclusion: Obesity and hepatic steatosis significantly favor the development of colorectal cancer liver metastases and the individual adipocytokines genetic profile may play an important predictive role.*

Correspondence to: Institutional BioBank, Experimental Oncology and Biobank Management Unit, IRCCS Istituto Tumori "Giovanni Paolo II", V.Le Orazio Flacco, 65 -70124, Bari, Italy. Tel: +39 805555259, e-mail: rosadive@inwind.it

Key Words: Adiponectin, leptin, TNF-α, colorectal cancer, metabolic syndrome, obesity, NAFLD.

Currently, obesity and the related conditions, including metabolic syndrome (MetS), represent an important risk factor for colorectal cancer (CRC), which have led to an increased incidence for this type of cancer. Non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of the MetS, is an additional risk factor for liver metastasis of colorectal cancer (1-3). Moreover, given its highly favorable microenvironment for the onset of liver metastases, NAFLD represents a negative prognostic factor in patients with CRC (4). Visceral obesity is one of the factors that predispose to NAFLD and the accumulation of fat both at visceral and hepatic level leads to a dysfunction of the adipose tissue and in an imbalance of adipocytokines profile (5). The state of chronic inflammation caused by fat accumulation induces an increase in pro-inflammatory cytokines, such as tumor necrosis factor alfa (TNF-α), and a reduction of the adiponectin levels (ADIPOQ), an adipocytokine with anti-inflammatory action (6, 7). An increased Body Mass Index (BMI), insulin resistance, and the presence of NAFLD, all features of MetS, sustain the inflammatory state, which is a fertile soil for the onset of cancer. In fact, MetS induces a state of insulin resistance and chronic inflammation with a consequent increase in the expression of the triad of pro-inflammatory cytokines TNF-α, Interleukin-1 (IL-1) and interleukin-6 (IL-6), associated with of a significant release of C-reactive protein (CRP) into the blood circulation (8-10). However, in obese subjects, adipocyte dysfunction is associated with a decreased ADIPOQ release into the circulation, with lack of insulin sensitization, and anti-inflammatory, anti-steatosic and antitumor actions (11). Regardless of other concomitant factors, the expansion of the adipocyte burden deprives patients with NAFLD of the anti-inflammatory and anti-fibrotic effect exerted by adiponectin

whose levels are inversely correlated to the increase in adiposity (12, 13). Another adipocytokine that is involved in the pathogenesis of NAFLD is Leptin. Leptin has a dual role in NAFLD (14). First, it plays a protective role against hepatic steatosis, especially at the early stages of the disease, and second, it exerts a non-beneficial effect by acting as a pro-inflammatory and fibrinogenic adipocytokine. Leptin exerts antisteatosic effects acting at the hepatocyte level (15, 16). A state of leptin resistance appears if expansion of the visceral fat mass occurs (17). Accordingly, leptin no longer compensates for insulin resistance thus losing its antisteatosic action (18). Unhealthy eating habits and poor physical activity represent the two main environmental risk factors that predispose to obesity and related conditions (*i.e.* MetS, NAFLD and insulin resistance). Notably, environmental factors may act on a genetic predisposition, and interactions between environmental factors and inter-individual genetic variations could result in a more aggressive phenotype of the disease (19, 20). *ADIPOQ* gene, which encodes for adiponectin, is located on chromosome 3q27 and is linked to a susceptibility locus for MetS. Circulating levels of ADIPOQ are influenced by genetic components (21). Among the variations of *ADIPOQ* gene, the SNP rs266729 found within the promoter region is significantly associated with ADIPOQ level and linked to susceptibility to cancer (22). ADOPOQ has been proposed as a determinant factor in the etiology of the MetS, because of its important regulatory action on insulin sensitivity and inflammation (23). Thus, the polymorphism in the *ADIPOQ* gene is believed to play a role in the pathogenesis of the MetS. Obesity and insulin resistance may be influenced by polymorphisms in Tumor necrosis factor α gene (*TNFA*) (24). The polymorphism *TNFA*-308 A/G in the gene promoter is associated with increased serum TNF-α levels and predisposes to insulin resistance (25, 26). It has been shown that individuals who carry the 308A *TNFA* gene variant are at higher risk of developing obesity compared to controls and show significantly higher systolic arterial blood pressure and plasma insulin levels (27). This supports the hypothesis that TNFA gene could be involved in the pathogenesis of the metabolic syndrome (28). Also, genetic polymorphisms in the promoter region of TNFA gene are associated with different inflammatory and malignant conditions (29-31). In this study, we evaluated whether the altered profile of adipocytokines and the genetic fingerprint of the NAFLD-associated metabolic syndrome "cluster" represent synergistic risk factors for predicting the onset of liver colorectal cancer metastases.

Materials and Methods

Patients. In this study, from June 2014 to December 2017, 165 colorectal cancer patients were enrolled at IRCCS (National Cancer Research Institute) Giovanni Paolo II of Bari –Italy. In addition, a group of 50 healthy subjects was included. All patients diagnosed with colon cancer had positive colonoscopy, which was confirmed histologically. The clinical characteristics of patients (age, sex, therapeutic interventions, *etc*.) were obtained from medical records. At the time of enrollment, of the 165 patients, 62 (37.5%) were non-metastatic patients, 69 (41.8%) had liver metastases and 34 (20.6%) had lung metastasis. In all subjects, body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Waist circumference was measured in centimeter up to the umbilicus in the standing position after normal expiration. Metabolic syndrome (MetS) was also determined for all participants. Patients were considered to have MetS when they presented 3 or more of the joint statement criteria of the American Heart Association/National Heart Lung and Blood Institute (AHA/NHLBI) and the International Diabetes Federation (IDF). The presence of NAFLD in all participants independent from alcohol consumption was determined through radiological evidence of hepatic steatosis. In our series, 93 (56.3%) patients with metabolic syndrome were all associated with the presence of NAFLD. Insulin resistance was calculated according to the homeostasis model assessment-Insulin Resistance (HOMA IR). This score is used to define insulin resistance in research. HOMA-IR=(fasting glucose in mmol/l × fasting insulin)/22.5 OR (fasting glucose in mg/dl × fasting insulin)/405. HOMA-IR ≥2.50 indicates insulin resistance.

Blood collections. All blood samples of cancer patients were obtained preoperatively or prior to other therapeutic procedures. For all participants, venous blood samples were collected after an overnight fast, and serum samples were either used immediately for analysis of the biochemical profile (glucose, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) cholesterol and triglycerides, ALT, AST, GGT, insulin, CRP), or were stored frozen at –20˚C for subsequent adipocytokine ELISA assay.

ADIPOQ, Leptin and TNF-a enzyme-linked immunoassay (ELISA). Serum samples from 165 CRC patients and 50 healthy donors were assayed for the levels of ADIPOQ, leptin and TNF-α by a sandwich ELISA assay (Quantikine Human ADIPOQ, Leptin and TNF-alfa Immunoassay; R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's recommendations. The absorbance of the solution produced was measured at 490 nm and was directly proportional to the amount of ADIPOQ, Leptin and TNF-alfa present in the sample. A standard curve was constructed by plotting the mean absorbance value measured for each standard *versus* its corresponding concentration.

Genotyping. Genotyping was performed after extraction of DNA from whole blood in EDTA using a standard QIAmp kit (QIAGEN Inc.) following the manufacturer's recommendations. DNA was dissolved in (TE) buffer and quantified by measurement of optical density at 260 nm. To improve the genotyping quality and validation, all mutant and heterozygous samples were re-genotyped, and results were noted only for those samples that were reproducible and with no discrepancy.

ADIPOQ rs266729 polymorphism. For detection of the adiponectin rs266729 polymorphism, we performed a Tetra amplification refractory mutation system polymerase chain reaction (T-ARMS-PCR) as previously reported (22). Briefly, two external primers and

Table I. *Association between adipocytokines, insulin and CRP profile with clinical characteristic of CRC patients.*

	N (%)	TNF-α pg/ml	*p*-Value	ADIPOQ ng/ml	*p*-Value	Leptin pg/ml	*p*-Value	Insulin pg/ml	*p*-Value	PCR	*p*-Value
Control group	50	48.1±2.5	0.000	160±35	0.000	6630±98	0.000	2.5±2.5	0.000	2.5±2.5	0.000
Patients group	165	72.2±40.9		103.0±96.9		28410±17905		22.6±23		9±8	
Gender											
Males	95 (57.5)	58.9±49.9	0.004	111.8±97	0.3	14648±13610	0.001	24±22	0.000	7±6.5	0.000
Females	70 (42.4)	87.5±77		96.6±95.2		23109±19840		18.6±16		11.5±9	
Tumor location			0.05		0.000		0.6		0.1		0.1
Colon	60 (41.3)	100±48		77±21		18365±17236		8.5±6.3		8.5±6	
Sigma	43 (29.6)	95.8±53.9		84.5±25		15489±+13256		9.1±3.2		9±3	
Rectum	62 (42.7)	118±51		58.6±36		17896±15265		10.1±3.5		10±3.5	
Grading			0.000		0.8		0.4		0.003		0.003
G1	45 (27.2)	75±55		80±42.4		19228±18850		24±20		11±10	
G2	63 (38.1)	119±89		75±69		24112±19759		25±21		11±9	
G3	57 (34.5)	60.8±53.5		73.9±44.3		15162±12753		20±20		6.4±5	
Stage			0.05		0.8		0.1		0.000		0.000
I-II	37 (22.4)	61.7±52.5		89±88		14637±12469		26±18		9±8	
III-IV	128 (77.5)	87±60		86±84		19489±17764		23±10		18±10	
Lymph node status			0.7		0.3		0.2		0.1		0.1
N0	54 (32.7)	86±78		100±94.4		17963±17087		26±26		8±8	
N1/N2	111 (67.2)	82±75		86±85		21383±17393		26±21		10±8.2	
Metastases			0.001		0.000		0.008		0.000		0.000
M0	62 (68.4)	74±72		135±91		17801±17187		22±21		8±7	
M1	103 (31.5)	114±85		68±58		25320±18593		23±20		13±8.6	
Metastatic sites			0.0001		0.001		0.01		0.01		0.001
Liver	69 (66.9)	185±20		45±25		32000±15000		27±18		15.5±7	
Lung	34 (33.1)	110±30		62±20		23520±20000		18±15		10±8	
BMI (kg/mq)			0.000		0.000		0.000		0.000		0.000
≤24.9	45 (27.2)	25±21.9		225.5±70.8		4586±1777		5.3±2.4		3.3±2.4	
≥25≤29.9	75 (45.4)	62.5±59.7		68.6±30.9		12433±7011		14.9±10		6.9±5.5	
≥30	45 (27.2)	151.2±71.2		40.5±24		47252±8341		33.8±14.5		18.8±6.5	
MetS/NAFLD			0.000		0.000		0.000		0.000		0.000
Yes	93 (56.3)	107.5±85.2		47.4±40.4		27300±18640		32.5±24		11.6±8.7	
No	72 (43.6)	37.6±35.6		169.6±99.7		7441±6251		6.5±4.3		4.7±4.4	
HOMA IR			0.004		0.000		0.000		0.000		0.000
≤2.5	88 (53.3)	58.5±48.7		177.8±67.9		11204±10000		4.4±2.5		6.5±6.4	
>2.5	77 (46.6)	87.2±74.7		96.2±81.3		36909±20035		33.1±27		12.2±8.6	

two specific internal allele specific primers were used: external primers (Forward outer: 5'-GGA CTG TGG AGA TGA TAT CTG GGG GGC A-3', Reverse outer: 5'-TGG CCT AGA AGC AGC CTG GAG AAC TGG A-3'), and allele specific internal primers (Forward inner (C allele): 5'-CTT GCA AGA ACC GGC TCA GAT CCT CCC- 3', Reverse inner (G allele): 5'-GAG CTG TTC TAC TGC TAT TAG CTC TGC-3'). The final PCR mixture (20 μl), contained DNA (2 μl), 10 × PCR buffer (1.5 μl), 2 mM MgCl2 (1.5 μl), 10 mM dNTP (0.3 μl), 0.25 μl of each primer 1 U Taq DNA polymerase and water. The reaction cycle consisted of pre-denaturation at 95˚C for 2 min, denaturation at 95˚C for 20 sec, 35 cycles of annealing at 56˚C for 20 sec, extension at 72˚C for 40 sec and a final extension at 72˚C for 4 min for complete extension of all PCR fragments. The reaction was performed on a Thermal Cycler, BioRAD (Milan, Italy). The amplified DNA fragments were verified on a 2% agarose gel. Each study participant was classified into one of the three possible genotypes: homozygote C/C, heterozygote C/G or homozygote G/G.

Genotyping of TNFA -308A/G gene polymorphism. The genotyping was performed using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique. Amplification of the -308 *TNFA* gene polymorphic region was performed in a Thermal Cycler, BioRAD, using the following primers (Invitrogen Life Technologies, Carlsbad, CA, USA): 5'-AGG CAA TAG GTT TTG AGG GCC AT-3' (Forward) and 5'-TCC TCC CTG CTC CGA TTC CG-3' (Reverse). To introduce a restriction site into the wild-type nucleotide sequence during the amplification reaction, the forward primers for each *TNFA* gene polymorphism contain a single base-pair mismatch adjacent to the polymorphic site. The PCR was carried out in a final volume of 12.5 μl containing 0.1 μg/μl of DNA, 3 mM of each primer, 0.025 U/μl of *Taq* DNA polymerase, 1.25 μl of supplied buffer enzyme 1X, 2.5 mM of $MgCl_2$, and 2.5 mM of each dNTP (Invitrogen Life Technologies, Carlsbad, CA, USA). Amplification conditions were as follows: initial denaturation at 94˚C for 4 min, 33 cycles of 94˚C for 30 sec, 60˚C for 30 sec and 72˚C for 30 sec, followed by 72˚C for 2 min for ending extension; resulting

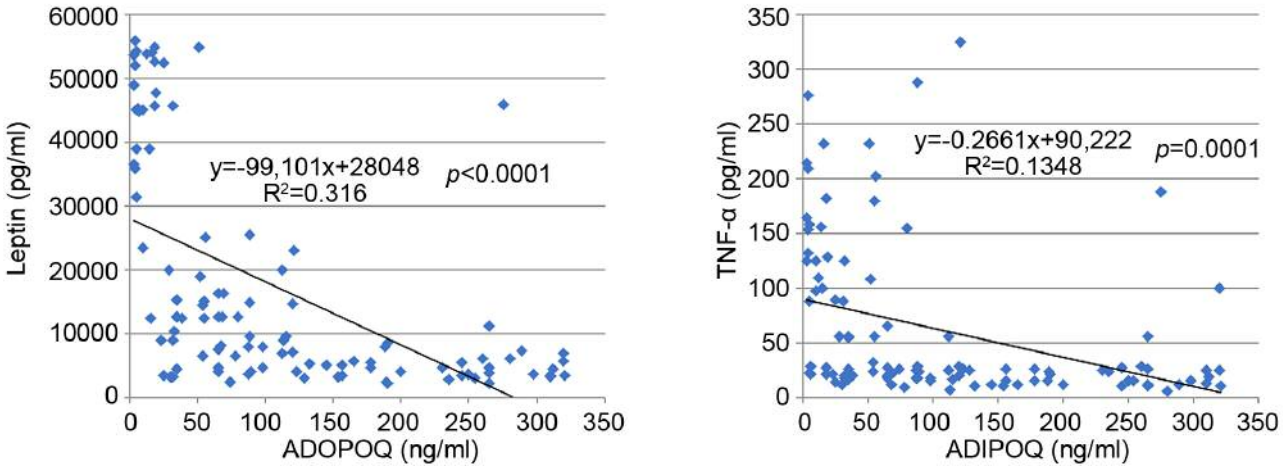


Figure 1. *Scatter plot showing an inverse correlation between serum levels of adiponectin with leptin and TNF-alpha.*

fragments of 107 bp was analyzed on a 6% polyacrylamide gel stained with silver nitrate (Invitrogen Life Technologies, Carlsbad, CA, USA). The amplified fragments of the -308 *TNFA* gene SNPs were incubated with 3 U *NcoI* restriction enzymes (New England BioLabs, Beverly, MA, USA), for 1 h at 37˚C. Finally, the digested PCR products were electrophoresed on 6% polyacrylamide gels and stained with silver nitrate for genotype identification. For the -308 SNP, fragments of 87 and 20 bp represent the wild-type genotype (G/G), fragments of 107, 87 and 20 bp represent the heterozygote genotype (G/A), and only one fragment of 107 bp represents the homozygote genotype (A/A).

Statistical analysis. For continuous variables, data were analyzed with the Mann-Whitney *U*-test, the unpaired Student's *t*-test, ANOVA and Fischer's exact test. Spearman's correlation was used for correlation analysis between serum adipocytokine profiles. The allelic and genotypic frequencies were estimated by the Chi-square test and Fisher's Exact test. Odds ratios (ORs) and 95% confidence intervals (95%CI) were calculated from the logistic model. *p*-Values ≤0.05 were considered statistically significant. All statistical analyses were performed by the Number Cruncher Statistical System-Power Analysis and Sample Size Software 2007 (NCSS-PASS, 329 North 1000 East Kaysville, UT, USA).

Results

Association between adipocytokines, Insulin, and CRP profile with clinical characteristic of CRC patients. Serum levels of Adiponectin, Leptin, TNF-α, Insulin and CRP showed statistically significant differences ($p<0.00001$, *t*-test) between the control group and the patients, Table I. This suggests that these adipocytokines are actively involved in the promotion and progression of CRC. Serum levels of adiponectin were decreased compared to other adipocytokines, which instead were generally increased. In fact, a statistically significant inverse correlation was observed between adiponectin and the other adipocytokines (*i.e.* leptin and TNF-α) as shown in Figure 1. Also, in patients with liver metastases, a notable reduction in adiponectin levels in the circulation with a consequent increase in the values of leptin, insulin, TNF-α and CRP was observed. The data suggest that liver is implicated in the regulation of circulating levels of all these parameters. It is worth mentioning that BMI, MetS/NAFLD, and insulin resistance significantly influences serum levels of adipocytokines. Furthermore, patients with BMI >30, with MetS/NAFLD and a state of insulin resistance showed a greater risk of developing liver metastases (Table II). These findings suggest that the triple condition of BMI, MetS/NAFLD and HOMA IR may constitute a unique phenotype that exposes the patient to a favorable microenvironment for the development of liver metastases. Moreover, a condition of overweight/obesity is associated with the development of more advanced tumor stage ($p<0.0001$, Fisher's exact test), poorly differentiated tumors ($p=0.01$, Fischer's exact test), with lymph node involvement ($p<0.0001$, Fischer's exact test), and with the presence of metastases ($p<0.0001$, Fischer's exact test).

Association between Adiponectin and TNF-α genotypes with BMI, MetS/NAFLD and HOMA Index in CRC patients. To understand the serum patterns of adipocytokines, in the three groups of patients grouped by BMI, MetS/NAFLD and HOMA Index, the distribution of genotypes and alleles frequency of the *ADIPOQ* rs266729 C/G and *TNFA* 308 G/A polymorphism were investigated. We found that the presence of the *ADIPOQ* G allele were significantly higher in overweight (OR=5, $p=0.01$) and in obese patients (OR=6.7, $p=0.01$) than in normal weight patients suggesting that this genotype represents a risk factor for the development of obesity (Table III). Also, the presence of the G allele represents a significant risk factor for developing MetS

Table II. *Association between BMI, MetS/NAFLD and HOMA IR with clinical and biochemical characteristic in CRC patients.*

	BMI ≤24.9 (n=45)	BMI≥25≤29.9 (n=75)	BMI≥30 (n=45)	*p*-Value	MetS/ NAFLD yes (n=93)	MetS/ NAFLD no (n=72)	*p*-Value	HOMAIR≤2.5 N=88	HOMAIR>2.5 N=77	*p*-Value
Median age	68 (30-96)	70.5 (33-86)	6		68 (30-96)	70.5 (33-86)		68 (30-96)	70.5 (33-86)	
Gender				0.2*			0.1*			n.s.*
Male	19	30	21		59	37		56	40	
Female	36	40	19		34	35		32	37	
Stage				0.0001*			0.2*			n.s.*
T1-T2 (37)	23	8	6		24	13		22	15	
T3-T4 (128)	22	67	39		69	59		66	62	
Lymph node status				0.0001*			0.8*			n.s.*
N0 (54)	40	8	6		30	24		28	26	
N1-N2 (111)	5	67	39		63	48		60	51	
Metastases				0.00001*			0.00001*			0.00001*
Mo (62)	25	35	2		25	37		52	10	
Liver (69)	2	25	42		57	12		4	65	
Lung (34)	18	15	1		11	23		32	2	
Grading				0.01*			0.6*			0.05*
G1 (45)	23	17	5		25	20		23	22	
G2 (63)	16	26	21		33	30		31	32	
G3 (57)	16	27	14		35	22		34	23	
BMI	22±1.8	27±1.5	32.2±2	0.001**	123.7±49.5	94.8±36.6	0.001**	25±3.7	29±4.2	0.001**
TG	104.2±43.3	108±44.2	136.5±50	0.001**	42.9±14.8	48.7±12.8	0.009**	98.3±37	132.1±52.1	0.001**
HDL	47.9±13.3	44±13.8	37.8±21.4	0.01**	124.4±44.7	100.3±25	0.001**	47.3±14.1	43±13	0.04**
GLU	108.8±31	115.2±43.3	120±32.6		13.3±10	6.3±6.4	0.001**	94.1±11.1	139.84±7	0.001**
INS	8.88±8.4	9.4±9.3	17.5±8.3	0.001**	4.9±5.2	1.9±2.7	0.001**	4.4±2.5	19.84±6.89	0.001**
ALT	18.2±12.3	19.3±12.1	21.25±22		23.1±16.2	21.2±11.4		20.7±11.6	21.4±15.5	n.s.**
AST	21.1±15	23.2±14.7	19.3±8.6		55.7±34.1	42.6±37.5	0.02**	47.1±33.4	48.4±38.9	n.s.**
GGT	38.5±33.4	59.6±37.6	69.3±59.3	0.003**	3.2±0.6	3.2±0.7		6.9+3.1	9.95+3.94	0.001**

*p-values were calculated with Fischer exact test; **p-values were calculated with ANOVA test.

(OR=3.64, p=0.0000001) and insulin resistance (OR=5.7, p=0.01) (Tables IV and V). Moreover, analyzing the allelic distribution of *TNFA* polymorphisms, the presence of *TNFA* 308A allele was found to be significantly higher in overweight (OR=6.2, p=0.0001) and obese patients, (OR=4.2, p<0.00001). Also, the presence of the A allele represents a significant risk factor that predisposes to MetS/NAFLD and insulin resistance. Therefore, the genetic variability, the presence of the *ADIPOQ* G rs266729 and *TNFA* 308 A allele are associated with obesity, metabolic syndrome/NAFLD and insulin resistance. This genetic signature significantly influences the biochemical blood profile as reported below.

Association between ADIPOQ/TNFA polymorphisms with biochemical and clinical pathological characteristics of CRC patients. As shown in Table VI, the polymorphism 308 A/G in *TNFA* gene promoter is associated with increased serum TNF-α levels and predisposes to insulin resistance. In fact, this polymorphism is associated with increased blood levels of glucose and insulin (p=0.01, p<0.00001 respectively, ANOVA test). Furthermore, this polymorphism is associated with both high levels of GGT (p=0.002 and p<0.0001 respectively, ANOVA test) and to low levels of HDL (p=0.01 and 0.009 respectively, ANOVA test) which are two parameters indicative of metabolic syndrome and NAFLD. The rs266729 G/C *ADIPOQ* polymorphism is significantly associated with lower levels of circulating adiponectin (p<0.0001, ANOVA Test), and with increased insulin. Furthermore, these polymorphisms favor the establishment of an inflammatory microenvironment represented by elevated circulating levels of CRP, a well-established marker of inflammation. Moreover, leptin levels are also influenced by these polymorphisms. In fact, high levels of leptin are found in obese subjects, with insulin resistance and metabolic syndrome, suggesting that in these subjects a state of leptin resistance has been established. The development of leptin resistance is considered a hallmark of obesity. Moreover, these polymorphisms predispose to a more aggressive tumor phenotype with a poorly differentiated histotype, with involvement of lymph nodes and the presence of metastases.

Table III. *ADIPOQ C/G rs266729 and TNF-a 308 G/A genotypes and allele frequency in relation to BMI.*

	Normal weight (n=45)		Overweight (n=75)		Obese (n=45)		Normalweight vs. overweight OR (95%CI)	Normalweight vs. Obese OR (95%CI)
ADIPOQ	N	F	N	F	N	F		
CC (47)	25	0.55	15	0.20	7	0.15	Reference group	Reference group
CG (60)	11	0.24	35	0.46	14	0.31	5.3 (2.08, 13.46) p=0.0002	4.5 (1.43, 14.378) p=0.004
GG (58)	9	0.20	25	0.33	24	0.53	4.62 (1.71, 12.55) p=0.001	9.5 (3.6, 29.6) p=0.00005
Fenotype	N	F	N	F	N	F		
CC (47)	25	0.55	15	0.20	7	0.15	Reference group	Reference group
CG+GG (118)	20	0.44	60	0.80	38	0.84	5 (2.21-11.3) p=0.00005	1.35 (0.5-3.6) p=0.2
Allele	N	F	N	F	N	F		
C (94)	50	0.55	30	0.20	14	0.15	Reference group	Reference group
G (236)	40	0.44	120	0.80	76	0.84	5 (2.8-8.9) p=0.01	6.7 (3.35-13.7) p=0.01
TNF-α 308G/A	N	F	N	F	N	F		
GG (55)	28	0.62	15	0.20	12	0.26	Reference group	Reference group
GA (49)	12	0.26	14	0.18	23	0.51	2.1 (0.8-5.8) p=0.06	4.4 (1.7-11.8) p=0.001
AA (61)	5	0.11	46	0.61	10	0.22	17.1 (5.6-52.4) p=0.00001	4.6 (1.3-16.6) p=0.008
Fenotype	N	F	N	F	N	F		
GG (55)	28	0.62	15	0.20	12	0.26	Reference group	Reference group
GA+AA (110)	17	0.37	60	0.80	33	0.73	6.5 (2.8-15.05) p<0.00001	4.5 (1.8-11) p=0.0004
Allele	N	F	N	F	N	F		
G (110)	56	0.62	30	0.20	24	0.26	Reference group	Reference group
A (220)	34	0.37	120	0.80	66	0.73	6.2 (3.4-11.2) p=0.001	4.2 (2.2-8.09) p<0.00001

Table IV. *ADIPOQ C/G rs266729 and TNF-a 308 G/A genotypes and allele frequency in relation to MetS/NAFLD.*

	MetS/NAFLD no (n=72)		MetS/NAFLD yes (n=93)		MetS/NAFLD no *vs*. MetS/NAFLD yes OR (95%CI)
ADIPOQ	N	F	N	F	
CC (47)	34	0.47	13	0.13	Reference group
CG (60)	27	0.37	33	0.35	3.19 (1.4-7.2) p=0.002
GG (58)	11	0.15	47	0.50	11.1 (4.4-27.9) p=0
Fenotype	N	F	N	F	
CC	34	0.47	13	0.13	Reference group
CG+GG	38	0.52	80	0.86	5.5 (2.6-11.6) p<0.000001
Allele	N	F	N	F	
C	68	0.47	26	0.19	Reference group
G	76	0.52	106	0.80	3.64 (2.12-6.2) p<0.000001
TNF-α 308G/A	N	F	N	F	
GG (55)	40	0.55	15	0.16	Reference group
GA (49)	8	0.11	41	0.44	13.6 (5.2-35.7) p=0.00001
AA (61)	24	0.33	37	0.39	4.1 (1.87-9) p=0.0002
Fenotype	N	F	N	F	
GG (55)	40	0.55	15	0.16	Reference group
GA+AA (110)	32	0.44	78	0.83	6.5 (3.15-13.3) p=0.000
Allele	N	F	N	F	
G (110)	80	0.55	30	0.23	Reference group
A (160)	64	0.44	96	0.76	4 (2.3-6.7) p=0.000

Association between ADIPOQ rs266729 C/G and TNFA 308 A/G polymorphisms with liver and lung metastasis. The genotypes of these polymorphisms in relation to hepatic and lung metastases are shown in Figure 2. Results in Figure 2 show that the presence of CG or GG genotype in the *ADIPOQ* gene and the presence of GA or AA genotype in the *TNFA* gene represent risk factor for the onset of liver metastases (p=0.0001 for *ADIPOQ* genotype, p=0.002 for

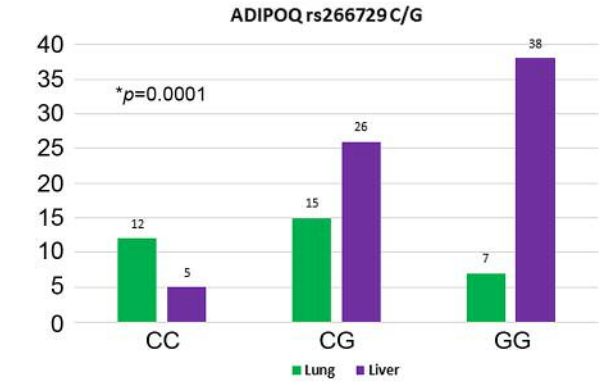
Table V. *ADIPOQ C/G rs266729 and TNF-a 308 G/A genotypes and allele frequency in relation to HOMA IR.*

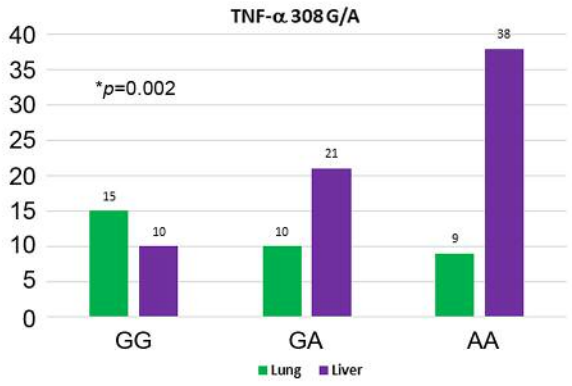
	HOMAIR≤2.5 N=88		HOMAIR>2.5 N=77		HOMAIR≤2.5 *vs*. HOMAIR>2.5 OR (95%CI)
ADIPOQ					
Genotype	N	F	N	F	
CC (47)	38	0.43	9	0.11	Reference group
CG (60)	30	0.34	30	0.38	4.2 (1.7-10.2) p=0.0007
GG (58)	20	0.22	38	0.49	2.8 (1.1-7.06) p=0.01
Fenotype	N	F	N	F	
CC	38	0.43	9	0.11	Reference group
CG+GG	50	0.56	68	0.88	5.7 (2.5-12.9) p=0.00001
Allele	N	F	N	F	
C	76	0.43	18	0.11	Reference group
G	100	0.56	136	0.88	5.7 (3.2-10.2) p=0.01
TNF-α 308G/A					
Genotype	N	F	N	F	
GG (55)	45	0.51	10	0.12	Reference group
GA (49)	25	0.28	24	0.31	4.3 (1.78-10.4) p=0.0005
AA (61)	18	0.20	43	0.55	10.7 (4.4-25.8) p=0.01
Fenotype	N	F	N	F	
GG	45	0.51	10	0.12	Reference group
GA+GA	43	0.48	67	0.87	7.01 (3.2-15.3) p=0.000001
Allele	N	F	N	F	
G	90	0.51	20	0.13	Reference group
A	86	0.48	131	0.86	6.8 (3.9-11.9) p=0.01

TNFA genotype; Fischer's exact test). These polymorphisms that affect the circulating levels of both adiponectin and TNF-α in these patients. In fact, there is a considerable decrease in adiponectin and an increase in TNF-α (particularly in patients harboring G allele in *ADIPOQ* gene and A allele in the *TNFA* gene) in CRC patients with liver metastases compared to those with lung metastases. Finally, Table VII shows a multiparametric analysis regarding the risk factors for the onset of liver metastases. Obesity, MetS/NAFLD, insulin resistance, the presence of the G allele in the adiponectin gene, low levels of adiponectin and high circulating levels of leptin and TNF-alpha generate a favorable microenvironment that predispose to development of liver metastases.

Discussion

Our study examined a more aggressive tumor phenotype in CRC patients with a greater risk of developing liver metastases. This phenotype is characterized by the presence of the following co-factors: BMI≥30, Homa IR≥2.5 and MetS/NAFLD. These patients generally show a decrease in serum levels of adiponectin and an increase in TNF-α and leptin circulating levels. This profile of circulating levels of adipocytokines is associated with a higher risk of developing liver metastases. Our findings corroborate the association of the adiponectin/leptin balance with the processes of mitogenesis, tumor growth and cell motility when adipose tissue dysfunction occurs (32, 33). We additionally investigated the influence of genetic variability of *ADIPOQ* and *TNFA* gene to understand the serum patterns of adipocytokines. In this study, we found lower levels of adiponectin in patients harboring the G allele in *ADIPOQ* gene and higher levels of TNF-alfa in patients harboring A allele in *TNFA* gene. *ADIPOQ* G/G rs266729 and *TNFA*-308 A/A genotype predispose to obesity state, to MetS/NAFLD and to insulin resistance. Our data are in accordance with a report by Hsieh CJ and co-workers, who have shown that the GG rs266729 *ADIPOQ* genotype predisposes to NAFLD and that this genotype significantly lowers serum levels of adiponectin, a condition that may worsen liver steatosis (34). In addition, another study by Suriyaprom K *et al*., has shown that decreased blood concentration of adiponectin is associated with GG *ADIPOQ* rs266729 polymorphism and that this is significantly more frequent in patients with metabolic syndrome (35). Our study clearly showed that this genetic susceptibility influences the biochemical blood profile in our patients. In fact, these polymorphisms are significantly associated with increased blood levels of glucose, insulin, HDL and GGT, thus favoring insulin resistance, the onset of metabolic syndrome and NAFLD. Our data agree with what has been reported by Nascimento H *et al*., who demonstrated





ADIPOQ	CC	CG	GG	**P-value
Liver (N=69)	98±40	55±45	25±20	0.0001
Lung (N=34)	110±45	88±55	78±50	0,3

TNF-a	GG	GA	AA	**P-value
Liver (N=69)	110±45	155±30	178±30	0.0001
Lung (N=34)	96±50	118±40	130±45	0,2

Figure 2. *Association between genetic polymorphism and serum profile of adiponectin and TNF-alpha with liver and lung metastasis in colorectal cancer patients. *p-Value calculated with Fisher's exact test; **p-value calculated with ANOVA test.*

Table VI. *Association between ADIPOQ/TNF-α polymorphisms with biochemical and clinical pathological characteristics of colorectal cancer patients.*

	ADIPOQ rs266729 C/G				TNF-α 308 G/A			
	CC (47)	CG (60)	GG (58)	*p*-Value	GG (55)	GA (49)	AA (61)	*p*-Value
Gender				0.33				0.2
Male (95)	29	30	36		30	33	32	
Female (70)	18	30	22		25	16	29	
Stage				0.4				0.5
T1-T2 (37)	12	10	15		15	10	12	
T3-T4 (128)	35	50	43		40	39	49	
Linph. status				0.4				0.9
N0 (54)	18	16	20		18	16	20	
N1/2 (111)	29	44	38		37	33	41	
Metastasis				<0.00001				0.002
M0 (62)	35	15	12		30	18	14	
M1 (103)	12	45	46		25	31	47	
Grading				0.0006				0.4
G1 (45)	18	20	7		20	12	13	
G2 (63)	15	28	20		19	20	24	
G3 (57)	14	12	31		16	17	24	
TG	121±13	124±65	141±85	0.2	126±60	132±67	120±35	0.5
HDL	44.1±17.4	37.1±15	35.2±17	0.01	44±14	38±18	47.4±17	0.01
GLU	109±31	109±27	127±60	0.03	110±46	117±25	128±20	0.01
INS	15.5±13	20±12	29±13	<0.00001	13±12.5	18.5±15	27±15	<0.0001
ALT	31.2±12	36.6±28	34±9.6	0.3	32.9±19	36.8±25	38.5±20	0.3
AST	27.6±12	28.8±18	30.5±15	0.6	27.8±20	32.2±18	33.2±12	0.1
GGT	53.4±46	56±49	57±45	0.9	45±7.8	70.2±32	58.3±51	0.002
LEPTIN	4868±2000	16055±7012	46000±9000	<0.00001	5868±3250	17522±12000	48520±8564	0.0001
TNF-A	85.6±50.2	135±34.6	153±19.2	<0.00001	88.5±45	136±30	158±20	0.0001
ADIPOQ	120.2±43.2	57.1±36.6	36±36	0.0001	115.3±40	66.5±30	35.5±30	0.0001
PCR	4.5±2.5	9±5.5	16.5±8	0.0001	3.2±2	7.5±6.5	18.5±8	0.0001

that adipokine gene SNPs are correlated with plasma levels of adipokine and lipid profile in pediatric obese patients (36). We reported that leptin levels are indeed influenced by *ADIPOQ* and *TNFA* polymorphisms, in fact, high levels of leptin are found in obese patients, with insulin resistance and MetS/NAFLD, indicating a consolidated state of leptin resistance. Leptin resistance is a hallmark of obesity (37). In obese individuals, elevated leptin acts as a pro-inflammatory adipokine and elevated circulating levels are associated with certain types of cancers (38). Leptin also stimulates the release of pro-inflammatory cytokines such as TNF-alpha, which in turn promotes inflammation and the overexpression of pro-angiogenic factors (*i.e.* VEGF and HIF-1α) with an increased risk for cancer development (39). Moreover, our study showed the establishment of a chronic inflammatory state sustained by high blood levels of CRP in patients with CRC. The association between central obesity, inflammation and insulin resistance, the three essential elements promoting NAFLD, led us to reinforce the hypothesis that NAFLD can be considered a hepatic manifestation of the metabolic syndrome, two conditions that share overlapping pathogenetic mechanisms (40, 41). The *primum movens* of pathogenesis is believed to be the relationship between insulin resistance and obesity. In the early stages, triglycerides accumulate in the hepatocyte with alterations in lipid metabolism. A central role is played by visceral adipose tissue (42, 43). High lipolytic activity of omental fat determines an increased flow of free fatty acids (FFA) that induce inflammatory pathways, leading to the development of both insulin and leptin resistance (44). In this scenario, adiponectin displays a down-regulatory effect in relation to weight gain, and it is possible that accumulation of visceral fat produces factors, such as TNF-α and leptin, that inhibit synthesis or secretion of adiponectin. The production of proinflammatory cytokines such as TNF-α at hepatic level and by macrophages of visceral adipose tissue appears to be an important mechanism (45, 46). TNF-α represents one of the earliest events of liver injury in NAFLD. TNF-α may cause insulin resistance in adipose tissue with an increased release of FFA in the blood circulation with the creation of a self-perpetuating vicious circle. In the liver, both hemodynamic and micro environmental processes are involved in trapping and killing circulating tumor cells. A failure at any step of these processes may favor the colonization of circulating tumor cells in the liver. A growing body of evidence suggests that adipocytes in the tumor microenvironment play a crucial role in disease progression by providing fatty acids, pro-inflammatory cytokines and proteases (47, 48). Since obesity is a well-recognized negative prognostic factor for colon cancer, increased adiposity may also have a negative effect in the treatment and survival of patients with CRC (49, 50). Therefore, a more appropriate anticancer therapy should consider treating concomitant conditions including metabolic syndrome, dyslipidemia, and insulin resistance. A reduction in body weight is essential. A 10% reduction in weight in overweight subjects can normalize transaminases and increase insulin sensitivity. Aerobic physical exercise and nutritional modifications can improve metabolic syndrome and NAFLD (51, 52). It is currently unclear whether obesity directly leads to metastatic disease *via* chronic systemic inflammation or whether obesity induces steatosis, which provides a fertile microenvironment for metastases. A combination of these factors is likely to occur. Indeed, our study showed that obesity and hepatic steatosis significantly favor the development of CRC liver metastases and that the individual genetic profile of adipocytokines may play an important predictive role.

Table VII. Multivariate analysis for prognostic parameter of liver metastasis occurrence.

	Liver metastasis (N=69)	Lung metastasis (N=34)	X^2	*p*-Value
Gender			0.006	0.93
Males	40	20		
Females	29	14		
Obesity			36.4	<0.0001
Yes	67	16		
No	2	18		
MetS/NAFLD			25.6	<0.0001
Yes	57	11		
No	12	23		
HOMA IR			78.1	<0.00001
Yes	65	2		
No	4	32		
ADIPOQ				
rs266729 C/G				0.0001
CC genotype	5	12	17	
CG genotype	26	15		
GG genotype	38	7		
Allele C	5	12	13	0.003
Allele G	64	22		
TNF-α A 308				0.002
GG genotype	10	15	12	
GA genotype	21	10		
AA genotype	38	9		
G allele	10	15	10	0.0009
A allele	59	19		
Adiponectin				0.00001
≥65	15	22	18	
<65	54	12		
TNF-α				
≥27.41	45	19	0.8	0.3
<27.41	24	15		
Leptin				
≥9556	50	13	11.2	0.0008
<9556	19	21		

Conflicts of Interest

The Authors have no conflicts of interest to declare regarding this study.

Authors' Contributions

Rosa Divella: Author of Project and Principal Investigator; Antonella Daniele: anthropometric parameters; Raffaele De Luca: Patients recruitment; Antonio Mazzocca: Statistical analysis; Eustachio Ruggieri: Patients recruitment; Eufemia Savino: biochemical blood profile; Porzia Casamassima: biochemical blood profile; Michele Simone: select the patients; Carlo Sabbà: Liver ultrasound for assessment of steatosis; Angelo Paradiso: oncological evaluation of patients.

Acknowledgements

The Authors would like to thank all nursing staff of the abdomen surgery unit for support in recruiting patients enrolled in this study. This study was supported by the research project "Quality control on biological samples stored in Biobank: prospective analysis of genetic variability and identifications of serum biomarkers predisposing to the metabolic syndrome in a series of patients with colon of the colon" financed by the Italian Ministry of Health.

References

1 Kabat GC, Kim MY, Stefanick M, Ho GYF, Lane DS, Odegaard AO, Simon MS, Bea JW, Luo J, Wassertheil-Smoller S and Rohan TE: Metabolic obesity phenotypes and risk of colorectal cancer in postmenopausal women. Int J Cancer *143(3)*: 543-551, 2018. PMID: 29488210. DOI: 10.1002/ijc.31345

2 Goulart A, Varejão A, Nogueira F, Martins S, Mesquita-Rodrigues A, Sousa N and Leão P: The influence of metabolic syndrome in the outcomes of colorectal cancer patients. Diabetes Metab Syndr *11(S2)*: S867-S871, 2017. PMID: 28711516. DOI: 10.1016/j.dsx.2017.07.007

3 Ahn JS, Sinn DH, Min YW, Hong SN, Kim HS, Jung SH, Gu S, Rhee PL, Paik SW, Son HJ and Gwak GY: Non-alcoholic fatty liver diseases and risk of colorectal neoplasia. Aliment Pharmacol Ther *45(2)*: 345-353, 2017. PMID: 27859470. DOI: 10.1111/apt.13866

4 Kondo T, Okabayashi K, Hasegawa H, Tsuruta M, Shigeta K and Kitagawa Y: The impact of hepatic fibrosis on the incidence of liver metastasis from colorectal cancer. Br J Cancer *115(1)*: 34-39, 2016. PMID: 27280634. DOI: 10.1038/bjc.2016.155

5 Divella R, Mazzocca A, Daniele A, Sabbà C and Paradiso A: Obesity, Nonalcoholic Fatty Liver Disease and Adipocytokines Network in Promotion of Cancer. Int J Biol Sci *15(3)*: 610-616, 2019. PMID: 30745847. DOI: 10.7150/ijbs.29599

6 Cao H: Adipocytokines in obesity and metabolic disease. J Endocrinol *220(2)*: T47-59, 2014. PMID: 24403378. DOI: 10.1530/JOE-13-0339.

7 Jung UJ and Choi MS: Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. Int J Mol Sci *15(4)*: 6184-223, 2014. PMID: 24733068. DOI: 10.3390/ijms15046184

8 Ebert T, Gebhardt C, Scholz M, Wohland T, Schleinitz D, Fasshauer M, Blüher M, Stumvoll M, Kovacs P and Tönjes A: Relationship between 12 adipocytokines and distinct components of the metabolic syndrome. J Clin Endocrinol Metab *103(3)*: 1015-1023, 2018. PMID: 29325128. DOI: 10.1210/jc.2017-02085

9 Sherling DH, Perumareddi P and Hennekens CH: Metabolic Syndrome. J Cardiovasc Pharmacol Ther *22(4)*: 365-367, 2017. PMID: 28587579. DOI: 10.1177/1074248416686187

10 Choi J, Joseph L and Pilote L: Obesity and C-reactive protein in various populations: a systematic review and meta-analysis. Obes Rev *14(3)*: 232-244, 2013. PMID: 23171381. DOI: 10.1111/obr.12003

11 Mather KJ and Goldberg RB: Clinical use of adiponectin as a marker of metabolic dysregulation. Best Pract Res Clin Endocrinol Metab *28(1)*: 107-117, 2014. PMID: 24417950. DOI: 10.1016/j.beem.2013.06.008

12 Adolph TE, Grander C, Grabherr F and Tilg H: Adipokines and non-alcoholic fatty liver disease: multiple interactions. Int J Mol Sci *18(8)* pii: E1649, 2017. PMID: 28758929. DOI: 10.3390/ijms18081649

13 Polyzos SA, Kountouras J and Mantzoros CS: Adipokines in nonalcoholic fatty liver disease. Metabolism *65(8)*: 1062-1079, 2016. PMID: 26725002. DOI: 10.1016/j.metabol.2015.11.006

14 Polyzos SA, Aronis KN, Kountouras J, Raptis DD, Vasiloglou MF and Mantzoros CS: Circulating leptin in non-alcoholic fatty liver disease: A systematic review and meta-analysis. Diabetologia *59*: 30-43, 2016. PMID: 26407715. DOI: 10.1007/s00125-015-3769-3

15 Rotundo L, Persaud A, Feurdean M, Ahlawat S and Kim HS: The Association of leptin with severity of non-alcoholic fatty liver disease: A population-based study. Clin Mol Hepatol *24(4)*: 392-401, 2018. PMID: 30068065. DOI: 10.3350/cmh.2018.0011

16 DI Maira G, Pastore M and Marra F: Liver fibrosis in the context of nonalcoholic steatohepatitis: the role of adipokines. Minerva Gastroenterol Dietol *64(1)*: 39-50, 2018. PMID: 28875689. DOI: 10.23736/S1121-421X.17.02427-8

17 Cernea S, Roiban AL, Both E and Huţanu A: Serum leptin and leptin resistance correlations with NAFLD in patients with type 2 diabetes. Diabetes Metab Res Rev *34(8)*: e3050, 2018. PMID: 30052309. DOI: 10.1002/dmrr.3050

18 Yadav A, Kataria MA, Saini V and Yadav A: Role of leptin and adiponectin in insulin resistance. Clin Chim Acta *417*: 80-84, 2013. PMID: 23266767. DOI: 10.1016/j.cca.2012.12.007

19 Meldrum DR, Morris MA and Gambone JC: Obesity pandemic: causes, consequences, and solutions-but do we have the will? Fertil Steril *107(4)*: 833-839, 2017. PMID: 28292617. DOI: 10.1016/j.fertnstert.2017.02.104

20 Sares-Jäske L, Knekt P, Lundqvist A, Heliövaara M and Männistö S: Dieting attempts modify the association between quality of diet and obesity. Nutr Res *45*: 63-72, 2017. PMID: 28967457. DOI: 10.1016/j.nutres.2017.08.001

21 Esfahani M, Movahedian A, Baranchi M and Goodarzi MT: Adiponectin: an adipokine with protective features against metabolic syndrome. Iran J Basic Med Sci *18(5)*: 430-442, 2015. PMID: 26124928.

22 Divella R, Daniele A, Mazzocca A, Abbate I, Casamassima P, Caliandro C, Ruggeri E, Naglieri E, Sabbà C and De Luca R: ADIPOQ rs266729 G/C gene polymorphism and plasmatic adipocytokines connect metabolic syndrome to colorectal cancer.

J Cancer *8(6)*: 1000-1008, 2017. PMID: 28529612. DOI: 10.7150/jca.17515

23 Lihn AS, Pedersen SB and Richelsen B: Adiponectin: action, regulation and association to insulin sensitivity. Obes Rev *6(1)*: 13-21, 2005. PMID: 15655035. DOI: 10.1111/j.1467-789X.2005.00159.x

24 Wen PF, Wang XS, Zhang M, Cen H, Pan HF, Ye QL, Mao C and Ye DQ: Associations between TNF gene polymorphisms (-308 A/G, -238 A/G, -1031 C/T and -857 T/C) and genetic susceptibility to T1D: a meta-analysis. Endocrine *46(3)*: 435-444, 2014. PMID: 24515539. DOI: 10.1007/s12020-014-0172-7

25 Gerasimova ON, Sigalovich EY, Dankovtseva EN, Nakonechnikov SN, Nikitin AG, Ivanova ZV, Masenko VP, Nosikov VV and Zateyshchikov DA: Carriage of a allele of polymorphic marker G(-238)A of TNF-alfa gene is associated with unfavorable prognosis in patients with chronic systolic heart failure. Kardiologiia *55(9)*: 25-30, 2015. PMID: 28294921.

26 Beecham J, Hart A, Alexandre L, Hernon J, Kumar B and Lam S: Single nucleotide polymorphisms and post-operative complications following major gastrointestinal surgery: A systematic review and meta-analysis. J Gastrointest Surg, 2019. PMID: 31270721. DOI: 10.1007/s11605-019-04300-2

27 Wu H, Yu K and Yang Z: Associations between TNF-α and interleukin gene polymorphisms with polycystic ovary syndrome risk: a systematic review and meta-analysis. J Assist Reprod Genet *32(4)*: 625-634, 2015. PMID: 25690158. DOI: 10.1007/s10815-015-0449-7

28 Sobti RC, Kler R, Sharma YP, Talwar KK and Singh N: Risk of obesity and type 2 diabetes with tumor necrosis factor-α 308G/A gene polymorphism in metabolic syndrome and coronary artery disease subjects. Mol Cell Biochem *360(1-2)*: 1-7, 2012. PMID: 22081334. DOI: 10.1007/s11010-011-0917-z

29 Sun Q, Guo X, Wang Q and Zhao F: The association of TNF-308 (G/A) gene polymorphisms and hepatocellular carcinoma risk: a meta-analysis. Chin J Cancer Res *28(5)*: 536-542, 2016. PMID: 27877013. DOI: 10.21147/j.issn.1000-9604.2016.05.09

30 Hu Q, Lou GG, Liu YC, Qian L and Lv BD: The Tumor Necrosis Factor-α-308 and -238 polymorphisms and risk of hepatocellular carcinoma for Asian populations: A meta-analysis. Curr Ther Res Clin Exp *76*: 70-75, 2014. PMID: 25352937. DOI: 10.1016/j.curtheres.2014.04.001

31 Sookoian SC, González C and Pirola CJ: Meta-analysis on the G-308A tumor necrosis factor alpha gene variant and phenotypes associated with the metabolic syndrome. Obes Res Dec *13(12)*: 2122-2131, 2005. PMID: 16421346. DOI: 10.1038/oby.2005.263

32 Parida S, Siddharth S and Sharma D: Adiponectin, obesity, and cancer: Clash of the Bigwigs in health and disease. Int J Mol Sci *20(10) pii*: E2519, 2019. PMID: 31121868. DOI: 10.3390/ijms20102519.

33 Modzelewska P, Chludzińska S, Lewko J and Reszeć J: The influence of leptin on the process of carcinogenesis. Contemp Oncol (Pozn) *23(2)*: 63-68, 2019. PMID: 31316286. DOI: 10.5114/wo.2019.85877

34 Hsieh CJ, Wang PW and Hu TH: Association of adiponectin gene polymorphism with nonalcoholic fatty liver disease in Taiwanese patients with type 2 diabetes. PLoS One *10(6)*: e0127521, 2015. PMID: 26042596. DOI: 10.1371/journal.pone.0127521

35 Suriyaprom K, Phonrat B and Tungtrongchitr R: Association of adiponectin gene -11377C>G polymorphism with adiponectin levels and the metabolic syndrome in Thais. Asia Pac J Clin Nutr *23(1)*: 167-173, 2014. PMID: 24561985. DOI: 10.6133/apjcn.2014.23.1.01

36 Nascimento H, Vieira E, Coimbra S, Catarino C, Costa E, Bronze-da-Rocha E, Rocha-Pereira P, Carvalho M, Ferreira Mansilha H, Rêgo C, Dos Santos R, Santos-Silva A and Belo L: Adipokine gene single-nucleotide polymorphisms in Portuguese obese adolescents: Associations with plasma concentrations of adiponectin, resistin, IL-6, IL-1β, and TNF-α. Child Obes *12(4)*: 300-313, 2016. PMID: 27159547. DOI: 10.1089/chi.2015.0235

37 Andreoli MF, Donato J, Cakir I and Perello M: Leptin resensitisation: a reversion of leptin-resistant states. J Endocrinol *241(3)*: R81-R96, 2019. PMID: 30959481. DOI: 10.1530/JOE-18-0606

38 Ghadge AA and Khaire AA: Leptin as a predictive marker for metabolic syndrome. Cytokine *121*: 154735. PMID: 31154250. DOI: 10.1016/j.cyto.2019.154735

39 Ghasemi A, Saeidi J, Azimi-Nejad M and Hashemy SI: Leptin-induced signaling pathways in cancer cell migration and invasion. Cell Oncol (Dordr) *42(3)*: 243-260, 2019. PMID: 30877623. DOI: 10.1007/s13402-019-00428-0

40 Buzzetti E, Pinzani M and Tsochatzis EA: The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism *65(8)*: 1038-1048, 2016. PMID: 26823198. DOI: 10.1016/j.metabol.2015.12.012

41 Karim MF, Al-Mahtab M, Rahman S and Debnath CR: Non-alcoholic fatty liver disease (NAFLD)--A Review. Mymensingh Med J *24(4)*: 873-880, 2015. PMID: 26620035.

42 Dietrich P and Hellerbrand C: Non-alcoholic fatty liver disease, obesity and the metabolic syndrome. Best Pract Res Clin Gastroenterol *28(4)*: 637-653, 2014. PMID: 25194181. DOI: 10.1016/j.bpg.2014.07.008

43 Lindenmeyer CC and McCullough AJ: The Natural History of Nonalcoholic Fatty Liver Disease-An Evolving View. Clin Liver Dis *22(1)*: 11-21, 2018. PMID: 29128051. DOI: 10.1016/j.cld.2017.08.003

44 Berk PD and Verna EC: Nonalcoholic Fatty Liver Disease: Lipids and Insulin Resistance. Clin Liver Dis *20(2)*: 245-262, 2016. PMID: 27063267. DOI: 10.1016/j.cld.2015.10.007

45 Boutari C, Tziomalos K and Athyros VG: The adipokines in the pathogenesis and treatment of nonalcoholic fatty liver disease. Hippokratia *20(4)*: 259-263, 2016. PMID: 29416297.

46 Polyzos SA, Aronis KN, Kountouras J, Raptis DD, Vasiloglou MF and Mantzoros CS: Circulating leptin in non-alcoholic fatty liver disease: a systematic review and meta-analysis. Diabetologia *59(1)*: 30-43, 2016. PMID: 26407715. DOI: 10.1007/s00125-015-3769-3.

47 Quail DF and Dannenberg AJ: The obese adipose tissue microenvironment in cancer development and progression. Nat Rev Endocrinol *15(3)*: 139-154, 2019. PMID: 30459447. DOI: 10.1038/s41574-018-0126-x

48 Cozzo AJ, Fuller AM and Makowski L: Contribution of Adipose Tissue to Development of Cancer. Compr Physiol *8(1)*: 237-282, 2017. PMID: 29357128. DOI: 10.1002/cphy.c170008

49 Shaukat A, Dostal A, Menk J and Church TR: BMI is a risk factor for colorectal cancer mortality. Dig Dis Sci *62(9)*: 2511-2517, 2017. PMID: 28733869. DOI: 10.1007/s10620-017-4682-z

50 Simkens LH, Koopman M, Mol L, Veldhuis GJ, Ten Bokkel Huinink D, Muller EW, Derleyn VA, Teerenstra S and Punt CJ: Influence of body mass index on outcome in advanced colorectal cancer patients receiving chemotherapy with or without targeted therapy. Eur J Cancer *47(17)*: 2560-2567, 2011. PMID: 21803570. DOI: 10.1016/j.ejca.2011.06.038

51 George ES, Forsyth A, Itsiopoulos C, Nicoll AJ, Ryan M, Sood S, Roberts SK and Tierney AC: practical dietary recommendations for the prevention and management of nonalcoholic fatty liver disease in adults. Adv Nutr *9(1)*: 30-40, 2018. PMID: 29438460. DOI: 10.1093/advances/nmx007

52 Romero-Gómez M, Zelber-Sagi S and Trenell M: Treatment of NAFLD with diet, physical activity and exercise. J Hepatol *67(4)*: 829-846, 2017. PMID: 28545937. DOI: 10.1016/j.jhep.2017.05.016

Received August 5, 2019
Revised August 20, 2019
Accepted August 21, 2019