Arch Gynecol Obstet (2015) 291:1147–1152
DOI 10.1007/s00404-014-3507-y

GYNECOLOGIC ONCOLOGY

L-Carnitine: a new insight into the pathogenesis of endometrial cancer

Dagistan Tolga Arioz · Mine Kanat-Pektas · Nadire Tuncer · Tulay Koken · Bekir Serdar Unlu · Gulengul Koken · Mehmet Yilmazer

Received: 26 November 2013 / Accepted: 7 October 2014 / Published online: 22 October 2014

Abstract
Objectives The present study aims to specify the role of L-carnitine in the pathogenesis of endometrial cancer by comparing the serum total L-carnitine levels of endometrial cancer patients with those of healthy women.
Methods Serum total L-carnitine concentrations were measured in patients with endometrioid-type endometrial cancer ($n = 20$) and healthy controls ($n = 20$) who were matched with respect to age and body mass index (BMI).
Results Stage I endometrial cancer was diagnosed in 12 women (60.0 %) whereas three women (15.0 %) had stage II disease, three women (15.0 %) had stage III disease and two women (10.0 %) had stage IV disease. The healthy controls and endometrial cancer patients were statistically similar in aspect of age, gravidity, parity, BMI, waist-to-thigh ratio, waist-to-hip ratio, menopause, complete blood count parameters, and serum biochemistry. Serum total L-carnitine levels of women with endometrial cancer were significantly lower than those of healthy women (respectively, 5,519.4 ± 2,712.5 vs 7,940.8 ± 3,566.6 ng/dl, $p = 0.021$). Moreover, serum total L-carnitine levels decreased significantly and progressively with advancing stage (stage I vs II vs III vs IV; 6,294.0 ± 2,885.1 vs 5,800.0 ± 441.2 vs 4,016.0 ± 2,833.3 vs 2,560.0 ± 67.9 ng/dl; $p = 0.021$).
Conclusions This is the first study to hypothesize that L-carnitine deficiency participates in the pathogenesis of endometrial cancer by means of a mechanism which is unrelated with obesity and increased amount of fat in human body.

Keywords Carcinogenesis · Endometrial cancer · L-Carnitine

D. T. Arioz · M. Kanat-Pektas (✉) · N. Tuncer · B. S. Unlu · G. Koken · M. Yilmazer
Department of Obstetrics and Gynecology, Medical School, Afyonkarahisar Kocatepe University, Marulcu Mah, Kadinana Cad. No: 22/4, 03200 Afyonkarahisar, Turkey
e-mail: minekanat@hotmail.com

T. Koken
Department of Biochemistry, Medical School, Afyonkarahisar Kocatepe University, Afyonkarahisar, Turkey

Introduction

Endometrial cancer is the most commonly encountered cancer of the female genital tract and the lifetime risk of being diagnosed with endometrial cancer is approximately 2.45 % (1 in 41) [1]. According to the American Cancer Society, 47,130 new cases of endometrium cancer were diagnosed and 8,010 deaths related with this disease occurred in 2012 [2]. These figures indicate that a notable success has not yet been achieved for survival from endometrial cancer despite the advancement in diagnostic tools and treatment options. Thus, recent research is directed towards the cellular signaling pathways and biochemical mechanisms which participate in the etiopathogenesis of the disease [3].

Carnitine is a naturally occurring compound which is synthesized from the amino acids lysine and methionine. This molecule is derived from either endogenous synthesis within hepatic and renal tissues or exogenous dietary sources such as red meat and dairy products. Its biologically active form is L-carnitine which is required for the transport of fatty acids from the cytosol into the mitochondria (where beta-oxidation enzymes are located for ATP production) during the breakdown of lipids [4]. Being primarily located within the mitochondria of living cells,

L-carnitine also possesses potential protective effects against many mitochondrial toxins [5].

Endometrial carcinoma is generally accepted to be estrogen responsive and the main risk factor for this disease is long-term exposure to excess endogenous or exogenous estrogen, without adequate opposition by a progestin. In other words, increasing age, early menarche and late menopause are associated with endometrial cancer. Other risk factors include obesity, nulliparity, diabetes mellitus, and hypertension [3]. It has been demonstrated that L-carnitine is depleted in elderly people and L-carnitine deficiency is related with obesity. Moreover, serum carnitine concentrations are lower in individuals with chronic diseases such as hypertension and diabetes mellitus [6, 7].

The present study aims to specify the role of L-carnitine in the pathogenesis of endometrial cancer by comparing the serum total L-carnitine levels of endometrial cancer patients with those of healthy women.

Materials and methods

Study design

This prospective case–control study was conducted at the department of obstetrics and gynecology in Afyon Kocatepe University Medical Faculty Hospital. The research protocol and consent procedures were approved by the Ethical Committee and Institutional Review Board of the study center and complied with the standards in the Declaration of Helsinki for Medical Research involving Human Subjects. All of the participants were informed about the study protocol and written informed consent was obtained from each subject.

Patients

The present study reviews 20 women who were sequentially diagnosed with endometrioid-type endometrial cancer and 20 healthy women who were admitted to the study center for menopause check up. The women with endometrial cancer and the healthy controls were matched with respect to age and body mass index. Endometrial cancer was diagnosed by the histopathological assessment of endometrial tissue specimens.

The age, gravidity and parity of the recruited subjects were recorded. After the height and weight of each participant were measured, body mass index (BMI) can be calculated by the following formula: Body mass index (kg/m^2) = Body weight (kg)/Height^2 (m^2).

Following revised FIGO staging, surgical staging was performed for every participant diagnosed with endometrial cancer [8]. All patients underwent surgical staging comprised of inspection of intraperitoneal cavity, peritoneal washing, total hysterectomy, bilateral salpingo-oophorectomy, and pelvic and para-aortic lymphatic sampling.

Exclusion criteria included the use of L-carnitine tablets as well as cardiovascular diseases, thyroid diseases, renal and hepatic dysfunction and concurrent or previous malignant disease. During the study period, three endometrial cancer patients and two healthy controls were diagnosed with diabetes mellitus and they were excluded from the study.

Laboratory studies

After clinical evaluation is finished, three blood samples were drawn from each participant by standardized phlebotomy technique. The first blood sample was designated to make complete blood count while the second blood sample was reserved for biochemical analysis. Complete blood count and biochemical analysis were accomplished by an automated analyzer (Cobas 6000 C501, Roche Applied Sciences, Basel, Switzerland) which was using commercially available diagnostic kits (Roche Diagnostics, Mannheim, Germany).

The remaining blood samples were reserved for the measurement of serum total L-carnitine levels which were determined before surgery was performed. These blood samples were centrifuged at 4,000 rpm for 10 min to remove cellular contents. Then the supernatants were collected and stored at −80 °C until the total L-carnitine levels were determined. After the frozen samples were thawed, serum total L-carnitine concentrations were measured by means of cytokine-specific and enzyme-linked immunosorbent assays (Cusabiotech Biotech, Wuhan, Hubei Province, China). The assays had a range of standards differing from 0.5 to 100.0 μmol/l. The inter- and intra-assay coefficients of variation were <5 % and, to avoid inter-assay variance, samples from endometrial cancer patients and healthy controls were measured in parallel and duplicate.

Statistical analysis

Collected data were analyzed by Statistical Package for Social Sciences version 18.0 (SPSS, SPSS Inc, Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation (range: minimum–maximum) whereas categorical variables were expressed as numbers or percentages. Smirnov–Kolmogorov test was used to test the data distribution. Independent samples *t* test and Mann–Whitney *U* test were utilized to compare the continuous and categorical variables of endometrial cancer patients and healthy controls. The alterations in serum total L-carnitine levels with respect to endometrial cancer stage were

Table 1 Demographic and clinical characteristics of endometrial cancer patients and healthy controls

	Endometrial cancer ($n = 20$)	Healthy controls ($n = 20$)	p
Age (years)	57.2 ± 12.8	50.1 ± 12.9	0.103
Gravidity	2.5 ± 1.0	2.8 ± 1.5	0.392
Parity	1.4 ± 0.7	1.5 ± 0.7	0.647
Height (m)	1.58 ± 0.06	1.60 ± 0.08	0.426
Body weight (kg)	93.8 ± 8.9	90.4 ± 9.6	0.252
Body mass index (kg/m^2)	37.8 ± 3.9	35.7 ± 4.8	0.144
Waist-to-thigh ratio	1.9 ± 1.1	1.6 ± 0.7	0.513
Waist-to-hip ratio	1.4 ± 0.8	1.2 ± 0.7	0.666
Post-menopause	18 (90.0 %)	16 (80.0 %)	0.661
Hypertension	3 (15.0 %)	0 (0.0 %)	0.019*

* $p < 0.05$ was accepted to be statistically significant

analyzed by one-way ANOVA test. Two-tailed p values <0.05 were accepted to be statistically significant.

A post hoc analysis was carried out to make a retrospective power analysis and it was determined that a cohort size of 40 women (20 healthy controls and 20 patients with endometrial cancer) had 67.6 % power to detect a difference at the 0.05 significance level.

Results

This study reviews 20 healthy women and 20 women with endometrioid-type endometrial tumor who were matched with respect to age and measures of obesity. Stage I disease was diagnosed in twelve women with endometrial cancer (60.0 %). Approximately 42 % of these women (5 out of 12) had stage IA disease whereas 58 % of them (7 out of 12) had stage IB disease. Three women (15.0 %) were diagnosed with stage II endometrial cancer while three women (15.0 %) had stage III disease and two women (10.0 %) had stage IV disease.

The healthy controls and endometrial cancer patients were statistically similar in aspect of similar age, gravidity, parity, height, body weight, BMI, waist-to-thigh ratio, waist-to-hip ratio and menopausal status (respectively, $p = 0.103$, $p = 0.392$, $p = 0.647$, $p = 0.426$, $p = 0.252$, $p = 0.144$, $p = 0.513$, $p = 0.666$ and $p = 0.661$). When compared with healthy controls, the frequency of hypertension was significantly higher in women with endometrial cancer ($p = 0.019$) (Table 1). The healthy controls and endometrial cancer patients had statistically similar complete blood count parameters, plasma glucose concentrations, lipid profiles, and hepatic and renal function tests ($p > 0.05$ for each) (Table 2).

Table 2 Laboratory data of endometrial cancer patients and healthy controls

	Endometrial cancer ($n = 20$)	Healthy controls ($n = 20$)	p
Hemoglobin (g/dl)	12.4 ± 1.4	12.5 ± 1.0	0.782
Leukocyte count ($\times 10^3$/mm^3)	10.0 ± 2.2	10.8 ± 2.7	0.319
Platelet count ($\times 10^3$/mm^3)	227.6 ± 72.5	199.1 ± 66.7	0.220
Urea (mg/dl)	6.7 ± 1.7	7.5 ± 3.2	0.299
Creatinine (mg/dl)	0.48 ± 0.11	0.51 ± 0.11	0.408
Glucose (mg/dl)	91.0 ± 23.9	92.5 ± 29.5	0.861
Alanine transferase (U/l)	13.4 ± 6.2	12.8 ± 4.8	0.708
Aspartate transferase (U/l)	18.7 ± 6.0	19.1 ± 5.0	0.876
Triglycerides (mg/dl)	299.2 ± 137.4	273.8 ± 116.9	0.533
Total cholesterol (mg/dl)	239.1 ± 41.6	241.8 ± 44.8	0.844
HDL (mg/dl)	56.3 ± 13.0	60.3 ± 12.1	0.317
LDL (mg/dl)	139.3 ± 30.8	152.3 ± 47.7	0.311
VLDL (mg/dl)	59.5 ± 27.6	55.6 ± 24.0	0.636

* $p < 0.05$ was accepted to be statistically significant

Serum L-carnitine concentrations of women with endometrial cancer were found to be significantly lower than those of healthy women (respectively, 5,519.4 ± 2,712.5 vs 7,940.8 ± 3,566.6 ng/dl, $p = 0.021$). It was also found that serum L-carnitine levels decreased significantly and progressively with advancing stage (stage I vs II vs III vs IV; 6,294.0 ± 2,885.1 vs 5,800.0 ± 441.2 vs 4,016.0 ± 2,833.3 vs 2,560.0 ± 67.9 ng/dl; $p = 0.021$) (Fig. 1). There were no statistically significant correlations among age, gravidity, parity, height, measures of obesity and L-carnitine levels.

Discussion

Endometrioid histology is associated with unopposed estrogen, intraepithelial neoplasia with the endometrium, and younger patient age. A combination of hormonal factors and mutational events has been proposed as the cause of endometrioid-type endometrial cancer. That is, gene expression in the endometrium varies with the hormonal environment and the genes that are most commonly expressed in secretory endometrium are scarcely found in endometrial carcinomas. From the standpoint of gene expression profiling, these neoplasms resemble

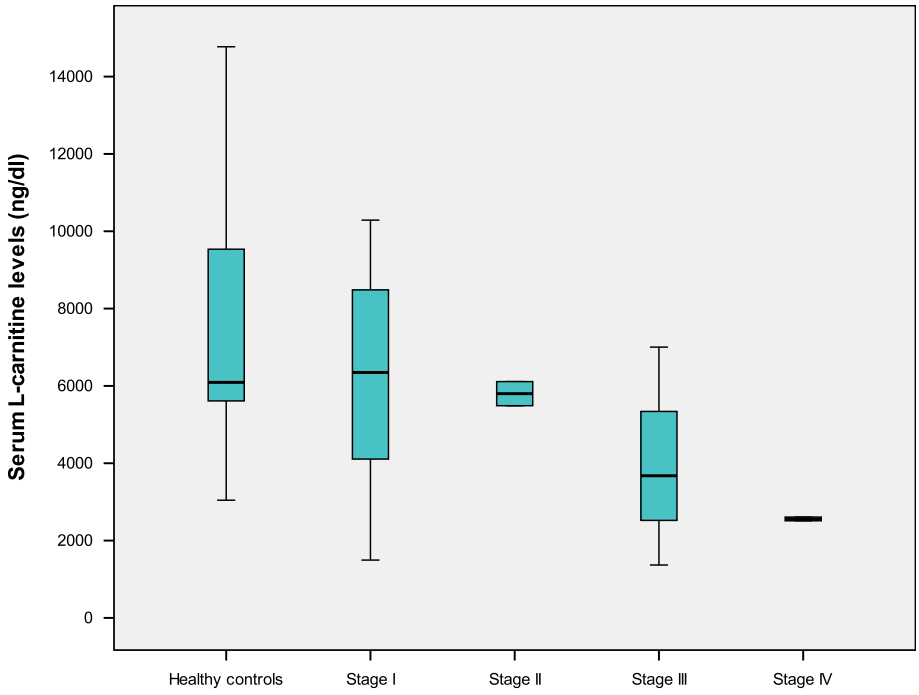

Fig. 1 Serum L-carnitine levels of healthy controls and endometrial cancer patients with respect to stage

proliferative endometrium rather than secretory epithelium. This may explain, in part, the anti-neoplastic effect of progestins [9].

The common genetic abnormalities identified in endometrioid-type endometrial tumors include microsatellite instability, K-ras and PTEN mutations, and defects in DNA mismatch repair [10]. Loss of PTEN function appears to be an early event in the carcinogenesis of endometrioid endometrial adenocarcinomas, possibly suggesting the "gatekeeper" role for this gene [11]. Moreover, the expression of PTEN protein is reduced in normal endometrium exposed to unopposed estrogen, and this reduction becomes prominent with increasing age [12, 13]. As for p53 mutations, they occur infrequently in the late periods of development of endometrioid-type endometrial cancer [14].

About 20 % of sporadic endometrioid endometrial carcinomas demonstrate a molecular phenotype referred to as microsatellite instability (MSI). Microsatellites are short segments of repetitive DNA bases that are scattered throughout non-coding DNA. The MSI refers to the tendency to develop changes in the number of repeat elements due to DNA repair errors made during replication. This phenomenon is an early event in carcinogenesis due to inactivation of intranuclear proteins of the DNA mismatch repair system (i.e., MSH-2, MLH-1, and MSH-6). This defect is also seen in patients who have colon carcinoma associated with hereditary non-polyposis colorectal cancer syndrome [12–14].

Although L-carnitine has been widely marketed as a weight-loss supplement, no scientific evidence shows that it improves weight loss. However, oral L-carnitine intake reduces fat mass, increases muscle mass, improves lipid profile and attenuates fatigue [15, 16].

The updated literature has emphasized the importance of L-carnitine in the development of malignancies. Initially, it has been demonstrated that L-carnitine inhibits pre-neoplastic lesions and prevents the carcinogenesis within the hepatic tissues of Long Evans Cinnamon rats [17, 18]. It has been reported that mitochondrial dysfunction plays an essential role in the hepatocarcinogenesis via the production of reactive oxygen species (ROS). That is, L-carnitine is primarily located in mitochondria and the paucity of this compound may aggravate mitochondrial injury [19]. This finding is further supported by earlier studies which point out that carnitine deficiency should be viewed as an underlying risk factor in cisplatin-induced nephrotoxicity, cardiomyopathy and hepatotoxicity. These studies claim that carnitine effectively inhibits mitochondrial injury induced by ROS and mitochondria-dependent apoptosis [20–22]. In addition, administration of L-carnitine facilitates beta-oxidation so that the accumulation of free fatty acids and their toxic intermediates is inhibited, thus preventing their harmful effects on mitochondrial and cell membranes [5, 21].

Moreover, it was observed that L-carnitine administration efficiently suppressed the formation of neoplastic lesions in the mice that had 1,2-dimethylhydrazine (DMH)-induced colon cancer. Such an effect has been attributed to the annihilation of ROS or the reinforcement of DNA mismatch repair system within the colon epithelium [23].

A Polish study conducted by Gaudet et al. compared the serum metabolic profiles of 250 healthy women and 250 women with endometrial cancer. The levels of C5-acylcarnitines, octenoylcarnitine and decatrienoylcarnitine were reported to be significantly lower in women who had endometrial cancer. Nevertheless, the endometrial cancer patients and the healthy controls were not matched with respect to age, BMI and other measures of obesity [24].

To the best of our knowledge, this is the first study to hypothesize that L-carnitine deficiency participates in the pathogenesis of endometrial cancer by means of a mechanism which is unrelated with various measures of obesity. Since factors such as sex, age, nutritional status, and chronic diseases influence the serum concentrations of carnitine in human being, a case–control design was adopted for the present study. Therefore, endometrial cancer patients and healthy controls were matched with respect to sex, age, and the measures of obesity. Yet, the power of the present study is limited by the smaller cohort size, the lack of longitudinal data and the significantly higher frequency of hypertension in the endometrial cancer group.

The findings of the present study suggest that L-carnitine deficiency has a role in the pathogenesis of endometrial cancer and this association seems to be independent of obesity. This hypothesis can be supported by the significantly lower serum total L-carnitine levels in endometrial cancer patients when compared to the healthy controls who are matched with respect to the measures of obesity. The depletion of L-carnitine within the mitochondria of endometrial cells may cause a failure in the clearance of toxic metabolites and, thus, the accumulation of these metabolites may impair mitochondrial functions. Moreover, the diminution in L-carnitine concentration may trigger a disturbance in DNA repair systems operating within the endometrial cells. Both mechanisms may contribute to the carcinogenesis process. This presumption is also supported by the significant and progressive decrease in serum total L-carnitine level with concurrent advance in endometrial cancer stage. Thus, it can be suggested that long-term L-carnitine supplementation may be adopted to prevent, slow or reverse the occurrence of endometrial tumors.

In vivo studies are warranted to clarify the probable association between L-carnitine deficiency and endometrial carcinomas. Furthermore, randomized placebo-controlled studies are required to determine whether nutritional supplementation of L-carnitine would help to gain to control over endometrial carcinogenesis.

Conflict of interest The authors declare that there are no conflicts of interest.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ (2008) Cancer statistics. CA Cancer J Clin 58(2):71–96
2. Siegel R, Naishadham D, Jemal A (2012) Cancer statistics. CA Cancer J Clin 62(1):10–29
3. Shu J, Fang S, Teichman PG, Xing L, Huang H (2012) Endometrial carcinoma tumorigenesis and pharmacotherapy research. Minerva Endocrinol 37(2):117–132
4. Steiber A, Kerner J, Hoppel C (2004) Carnitine: a nutritional, biosynthetic, and functional perspective. Mol Aspects Med 25(5–6):455–473
5. Arrigoni-Martelli E, Caso V (2001) Carnitine protects mitochondria and removes toxic acyls from xenobiotics. Drug Exp Clin Res 27(1):27–49
6. Steiber A, Kerner J, Hoppel C (2004) Carnitine: a nutritional, biosynthetic, and functional perspective. Mol Aspects Med 25(5–6):455–473
7. Sahlin K (2010) Boosting fat burning with carnitine: an old friend comes out from the shadow. J Physiol 589(Pt 7):1509–1510
8. Pecorelli S (2009) Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynecol Obstet 105(2):103–104
9. Mutter GL, Baak JP, Fitzgerald JT, Gray R, Neuberg D, Kust GA et al (2001) Global expression changes of constitutive and hormonally regulated genes during endometrial neoplastic transformation. Gynecol Oncol 83(2):177–185
10. Zhou XC, Dowdy SC, Podratz KC, Jiang SW (2007) Epigenetic considerations for endometrial cancer prevention, diagnosis and treatment. Gynecol Oncol 107(1):143–153
11. Mutter GL, Lin MC, Fitzgerald JT, Kum JB, Baak JP, Lees JA et al (2000) Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. J Natl Cancer Inst 92(11): 924–930
12. Mutter GL, Ince TA, Baak JP, Kust GA, Zhou XP, Eng C (2001) Molecular identification of latent precancers in histologically normal endometrium. Cancer Res 61(11):4311–4314
13. Lax SF (2004) Molecular genetic pathways in various types of endometrial carcinoma: from a phenotypical to a molecular-based classification. Virchows Arch 444(3):213–223
14. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I (2005) Endometrial cancer. Lancet 3668(9484): 491–505
15. Sahlin K (2010) Boosting fat burning with carnitine: an old friend comes out from the shadow. J Physiol 589(7):1509–1510
16. Wall BT, Stephens FB, Constantin-Teodosiu D, Marimuthu K, Macdonald IA, Greenhaff PL (2011) Chronic oral ingestion of L-carnitine and carbohydrate increases muscle carnitine content and alters muscle fuel metabolism during exercise in humans. J Physiol 589(4):963–973
17. Chang B, Nishikawa M, Nishiguchi S, Inoue M (2005) L-Carnitine inhibits hepatocarcinogenesis via protection of mitochondria. Int J Cancer 113(5):719–729
18. Al-Rejaie SS, Aleisa AM, Al-Yahya AA, Bakheet SA, Alsheikh A, Fatani AG et al (2009) Progression of diethylnitrosamine-induced hepatic carcinogenesis in carnitine-depleted rats. World J Gastroenterol 15(11):1373–1380
19. Singh KK (2004) Mitochondrial dysfunction is a common phenotype in aging and cancer. Ann N Y Acad Sci 1019(6):260–264
20. Chang B, Nishikawa M, Sato E, Utsumi K, Inoue M (2002) L-Carnitine inhibits cisplatin-induced injury of the kidney and small intestine. Arch Biochem Biophys 405:55–64
21. Furuno T, Kanno T, Arita K, Asami M, Utsumi T, Doig Y, Inoue M, Utsumi K (2001) Roles of long chain fatty acids and carnitine

in mitochondrial membrane permeability transition. Biochem Pharmacol 62:1037–1046
22. Hagen TM, Liu J, Lykkesfeldt J, Wehr CM, Ingersoll RT, Vinarsky V, Bartholomew JC, Ames BN (2002) Feeding acetyl-L-carnitine and lipoic acid to old rats significantly improves metabolic function while decreasing oxidative stress. Proc Natl Acad Sci USA 99:1870–1875
23. Roscilli G, Marra E, Mori F, Di Napoli A, Mancini R, Serlupi-Crescenzi O et al (2013) Carnitines slow down tumor development of colon cancer in the DMH-chemical carcinogenesis mouse model. J Cell Biochem 114(7):1665–1673
24. Gaudet MM, Falk RT, Stevens RD, Gunter MJ, Bain JR, Pfeiffer RM et al (2012) Analysis of serum metabolic profiles in women with endometrial cancer and controls in a population-based case-control study. J Clin Endocrinol Metab 97(9):3216–3223