Clinical Thyroidology / Original Paper

European
Thyroid Journal

Eur Thyroid J 2015;4:181–188
DOI: 10.1159/000437365

Received: February 26, 2015
Accepted after revision: June 30, 2015
Published online: August 20, 2015

Metformin Is Associated with a Favorable Outcome in Diabetic Patients with Cervical Lymph Node Metastasis of Differentiated Thyroid Cancer

Eun Kyung Jang[a, b] Won Gu Kim[b] Hyemi Kwon[b] Yun Mi Choi[b] Min Ji Jeon[b]
Tae Yong Kim[b] Young Kee Shong[b] Won Bae Kim[b] Eui Young Kim[a]

[a]Department of Endocrinology, Dongnam Institute of Radiological and Medical Sciences Cancer Center, Busan, and
[b]Division of Endocrinology and Metabolism, Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Key Words
Thyroid cancer · Neoplasm metastasis · Metformin · Diabetes mellitus · Recurrence

Abstract
Background and Objective: Type 2 diabetes is known to increase the risk and progression of certain types of cancer. Metformin treatment of diabetic patients is reported to have beneficial effects on some cancers. We evaluated the clinical outcome of diabetic patients with differentiated thyroid cancer (DTC) according to metformin treatment. ***Methods:*** We reviewed 943 patients diagnosed with DTC after total thyroidectomy between 1995 and 2005 in a tertiary hospital. The study involved 60 diabetic patients and 210 control patients matched for age, sex, body mass index (BMI), and tumor size. ***Results:*** There were no differences in the clinicopathological features and disease-free survival (DFS) between diabetic patients and the control group over 8.9 years of follow-up. Of the diabetic patients with DTC, 35 patients (58%) were treated with metformin. There were no differences in age, sex, BMI, tumor size, antidiabetic medication, glycated hemoglobin, or C-peptide levels in metformin and nonmetformin groups. However, cervical lymph node (LN) metastasis was more prevalent in the metformin group than in the nonmetformin group (OR 3.52, p = 0.035). Among diabetic patients with cervical LN metastasis of DTC, the metformin subgroup (17.1 years) was associated with longer DFS than the nonmetformin subgroup (8.6 years) (HR 0.16, p = 0.021); metformin treatment was also associated with longer DFS in this subgroup in multivariate analysis after adjusting age, BMI, duration of diabetes, presence of tumor at resection margin, and serum thyroglobulin level at ablation (HR 0.03, p = 0.035). ***Conclusions:*** Metformin treatment is associated with low recurrence in diabetic patients with cervical LN metastasis of DTC.

Introduction

Metformin has been reported to have an antineoplastic effect in certain cancers [1, 2]. It is the first-line antidiabetic medication in type 2 diabetes; it improves insulin sensitivity in peripheral organs and has a glucose-lowering effect. There are reports that cancer patients treated with metformin have better outcomes than patients not treated with metformin [3–6]. In breast cancer patients, metformin treatment has been associated with better overall survival [7]. In colorectal cancer, diabetic patients treated with metformin have also had 30% better overall survival than those receiving other diabetic medications [8].

Several experimental studies have reported on the antineoplastic effects of metformin on thyroid cancer cells

KARGER 125

E-Mail karger@karger.com
www.karger.com/etj

2235–0640/15/0043–0181$39.50/0

Eui Young Kim, MD
Department of Endocrinology
Dongnam Institute of Radiological and Medical Sciences Cancer Center
40 Jwadong-gil, Jangan-eup, Gijang-gun, Busan 46033 (Korea)
E-Mail euiykim@gmail.com

[9, 10]. The authors suggested that metformin activates adenosine monophosphate-activated protein kinase (AMPK) and inhibits the mammalian target of rapamycin pathway, which induces catabolism or apoptosis in cancer cells. In a recent study, it was suggested that metformin can reprogram cellular energy metabolism and cause cells adapted to the new environment to behave less aggressively [2].

Differentiated thyroid cancer (DTC) has a favorable prognosis, but some patients suffer a recurrence during follow-up [11]. Recurrence of DTC is associated with various factors including initial therapy, age, tumor size, gross soft tissue invasion, and distant metastasis [11, 12]. A previous study suggested that the use of metformin was an independent favorable prognostic factor because there was a significant association between metformin and remission rate in diabetic patients with thyroid cancer [9].

In this study, we compared clinical outcomes in diabetic patients with DTC and matched control DTC patients. To assess the metformin effect on DTC, we evaluated the clinical outcomes of diabetic patients with DTC according to metformin treatment. Finally, we compared the clinical outcomes of diabetic patients with cervical lymph node (LN) metastasis of DTC according to metformin treatment, and evaluated the effect of metformin on advanced DTC.

Materials and Methods

Patients

We retrospectively reviewed all patients diagnosed with DTC after total thyroidectomy between 1995 and 2005 in Asan Medical Center, Seoul, Korea. Patients aged between 45 and 75 years, with tumors between 1 and 4 cm, were included in the study. We excluded patients with distant metastasis before surgery or patients with type 1 diabetes. Among 943 DTC patients, we found 60 patients (6%) who had type 2 diabetes. The diagnosis of diabetes was confirmed if one or more of the following were present: (1) the patient had been treated with oral hypoglycemic agent or insulin before surgery or (2) there was documentation of hyperglycemia according to the diagnostic criteria for diabetes recommended by the World Health Organization in 2011, which included fasting plasma glucose ≥7.0 mmol/l, random plasma glucose ≥11.1 mmol/l, 2-hour plasma glucose ≥11.1 mmol/l during an oral glucose tolerance test, and glycated hemoglobin (HbA_{1c}) ≥6.5%. We defined type 1 diabetes based on age at diagnosis, body mass index (BMI), type of treatment, C-peptide level, and presence of autoimmunity such as glutamic acid decarboxylase antibody.

Finally, we randomly selected 210 patients matched for age, sex, BMI, and tumor size from the 883 nondiabetic patients with DTC as a control group. The study protocol was approved by the institutional review board of Asan Medical Center.

Definitions

Patients who had been treated with antidiabetic medication(s) for more than 6 months within 2 years after the thyroidectomy were defined as patients who had been treated with antidiabetic medication including metformin. We then divided those patients into a 'metformin group' and a 'nonmetformin group' according to whether or not they had received metformin treatment for more than 6 months within 2 years after thyroidectomy.

Clinical recurrence was defined as recurrence of structural disease such as the reappearance of pathologically proven malignant tissue and/or the appearance of metastatic lesions on imaging studies including neck ultrasonography, radioiodine whole body scan, computed tomography (CT) scan, and/or ^{18}F-fluorodeoxyglucose positron emission tomography with CT (PET-CT) at least 2 years after surgery.

Clinical Parameters

HbA_{1c} data were collected for 2 years after thyroidectomy and the mean HbA_{1c} over that period was used for analysis. We also collected serum C-peptide data within 2 years after surgery.

Follow-Up Protocols in the DTC Patients

All patients were treated with thyroxine to suppress thyroid-stimulating hormone and were regularly followed up with physical examinations, serum thyroglobulin (Tg), anti-Tg antibody measurement, and neck ultrasonography examinations every 6–12 months. A diagnostic whole body scan was performed during the first 6–24 months after initial therapy as previously described [13]. Additional diagnostic imaging studies including CT scan, magnetic resonance scan, and PET-CT were performed in some patients as needed. The median follow-up period was 8.9 years.

Statistical Analysis

R version 3.0 and the R libraries prodlim, car, Cairo, and survival were used for analyzing data and drawing graphs (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org). Continuous variables between two groups were compared using Student's t test. Categorical variables were compared using the χ^2 test or Fisher's exact test. A Cox proportional hazard model was used to evaluate the risks of recurrence. The multivariate analysis included age, sex, BMI, duration of diabetes, extrathyroidal extension, presence of tumor at resection margin, and serum Tg level at ablation. Disease-free survival (DFS) curves were constructed by the Kaplan-Meier method, and log-rank tests were used to evaluate differences of DFS between patient groups. All p values were two-sided, with $p < 0.05$ considered statistically significant.

Results

Baseline Characteristics of the Study Patients

Mean age of the patients was 58.3 ± 6.1 years, and 194 patients (72%) were female. Mean BMI at thyroid surgery was 25.2 ± 2.7. Of the 270 patients, 255 patients (94%) were diagnosed with the classical variant of papillary thyroid carcinoma and 7 patients (3%) were diagnosed with the follicular variant of papillary thyroid carcinoma. Five

Table 1. Comparison of the clinicopathological features of the diabetic patients and control group

	Total (n = 270)	Control (n = 210)	Diabetes (n = 60)	p
Age, years	58.3±6.1	58.2±5.7	58.7±7.1	0.63
Sex, female	194 (72)	173 (82)	44 (73)	0.14
BMI	25.2±2.7	25.1±2.8	25.6±2.3	0.13
Tumor size, cm	1.9±0.8	1.9±0.8	1.9±0.8	0.73
Histology				0.48
Classical PTC	255 (94)	199 (95)	56 (93)	
Follicular variant of PTC	7 (3)	5 (2)	2 (3)	
Follicular thyroid carcinoma	5 (2)	4 (2)	1 (2)	
Hürthle cell carcinoma	3 (1)	2 (1)	1 (2)	
Extrathyroidal extension	194 (72)	154 (73)	40 (67)	0.33
Tumor at resection margin	47 (17)	33 (16)	14 (23)	0.18
LN metastasis	144 (53)	114 (54)	30 (50)	0.56
N1a	116 (43)	91 (43)	25 (42)	
N1b	28 (10)	23 (11)	5 (8)	
Radioiodine remnant ablation	256 (95)	199 (95)	57 (95)	0.99
^{131}I dose at ablation, mCi	121±45	122±46	119±48	0.67
Tg at ablation, μg/l	7.6±32.3	7.6±32.7	7.7±29.3	0.99
Recurrence	39 (14)	33 (16)	6 (10)	0.31

Continuous variables including age, BMI, tumor size, ^{131}I dose, and serum Tg at ablation are reported as means ± SD; other values are n (%). PTC = Papillary thyroid carcinoma.

patients (2%) had follicular thyroid carcinoma and 3 patients (1%) had Hürthle cell carcinoma. There were no significant differences in histology, T stage, N stage, extrathyroidal extension, lymphovascular invasion, tumor at resection margin, or radioiodine remnant ablation between the diabetic patients and the control group (table 1).

Baseline Characteristics of Diabetic Patients

Mean duration between diagnoses of diabetes and thyroid surgery was 4.7 years (range: 0.5–21 years). Of the 60 diabetic patients, 35 patients (58%) were treated with metformin. Mean duration of metformin treatment was 7.4 ± 4.8 years, and the mean dose was 979 mg (range: 250–2,000). Thirty patients received 1,000 mg/day or less of metformin and 5 patients received over 1,000 mg/day of metformin. There was no difference in antidiabetic medication other than metformin between the metformin and nonmetformin groups. Mean HbA_{1c} was 7.3 ± 1.1%, and the mean C-peptide level was 3.5 ± 2.3 ng/ml. Cervical LN metastasis was more prevalent in the metformin group than in the nonmetformin group (OR 3.52, 95% CI: 1.08–12.37, p = 0.035; table 2). There were no significant differences between the groups in age, sex, BMI, preoperative thyroid-stimulating hormone values, the surgeons, extent of surgery, tumor size, HbA_{1c}, C-peptide, histology, T stage, extrathyroidal extension, lymphovascular invasion, tumor at resection margin, or radioiodine remnant ablation (table 2).

Clinical Outcomes in Diabetic Patients and the Control Group

Six of the 60 diabetic patients (10%), and 33 of the 210 controls (16%) suffered recurrence (table 1). In 6 diabetic patients with recurrence, 4 patients underwent additional surgery for recurrence, and 1 of these patients received radioactive iodine (RAI) treatment after surgery. Among 33 patients with recurrence in the control group, 26 patients underwent additional surgery for recurrence and 11 of these patients received RAI treatment after surgery. One patient from the control group received RAI treatment for recurred lesion without additional surgery. There was no significant difference in DFS between the two groups (p = 0.28; fig. 1a), and their rates of recurrence at distant organs were similar (p = 0.31).

Clinical Outcomes in the Metformin and Nonmetformin Groups

Three of the 35 patients (9%) in the metformin group, and 3 of the 25 (12%) in the nonmetformin group had recurrence (table 2). There was no difference in DFS be-

Table 2. Clinicopathological factors in the metformin and nonmetformin groups of diabetic patients

	Nonmetformin (n = 25)	Metformin (n = 35)	p
Age, years	60.4±7.2	57.5±6.9	0.12
Sex, female	17 (68)	27 (77)	0.56
BMI	25.5±2.4	25.7±2.2	0.68
Preoperative TSH values, mIU/l	1.7±0.9	1.9±2.6	0.21
Duration of diabetes, years	4.9±5.7	4.5±4.8	0.75
HbA_{1c}, %	7.3±1.0	7.3±1.1	0.92
C-peptide, ng/ml	3.0±1.9	3.8±2.6	0.37
Antidiabetic medication			
Insulin	2 (8)	1 (3)	0.57
Sulfonyluria	19 (76)	26 (74)	0.99
α-Glucosidase inhibitor	7 (28)	6 (17)	0.36
Thiazolidinediones	4 (16)	5 (14)	0.99
No medication	2 (8)	2 (6)	0.99
Surgeons			0.46
Surgeon A	16 (64)	26 (74)	
Surgeon B	4 (16)	5 (14)	
Surgeon C	3 (12)	4 (11)	
Surgeon D	2 (8)	0	
Extent of surgery			0.87
TT only	3 (12)	2 (6)	
TT with CND	20 (80)	30 (86)	
TT with MRND	2 (8)	3 (9)	
Tumor size, cm	1.9±0.9	1.8±0.8	0.78
Histology			0.99
Classical PTC	23 (92)	33 (94)	
Follicular variant of PTC	1 (4)	1 (3)	
Follicular thyroid carcinoma	1 (4)	0	
Hürthle cell carcinoma	0	1 (3)	
Extrathyroidal extension	16 (64)	24 (69)	0.78
Tumor at resection margin	8 (32)	6 (17)	0.22
LN metastasis	8 (32)	22 (63)	0.035
N1a	6 (24)	19 (54)	
N1b	2 (8)	3 (9)	
Radioiodine remnant ablation	24 (96)	33 (94)	0.99
^{131}I dose at ablation, mCi	125±42	124±42	0.97
Tg at ablation, μg/l	12.9±43.5	4.1±11.3	0.34
Recurrence	3 (12)	3 (9)	0.69

Continuous variables including age, BMI, time interval between diagnosis of diabetes and surgery, HbA_{1c}, C-peptide, tumor size, ^{131}I dose, and serum Tg at ablation are reported as means ± SD; other values are n (%). HbA_{1c} data were available for 49 patients (78%): 31 patients (72%) in the metformin group and 18 (86%) in the nonmetformin group. C-peptide was measured in 31 patients (52%) including 18 patients (51%) in the metformin group and 13 (52%) in the nonmetformin group. TT = Total thyroidectomy; CND = central neck dissection; MRND = modified radical neck dissection; PTC = papillary thyroid carcinoma; TSH = thyroid-stimulating hormone.

tween the two groups (p = 0.72; fig. 1b). There was also no difference in recurrence at distant organs (p = 0.99). When we compared the recurrence rate and DFS according to the dose of metformin (1,000 mg/day or less, and over 1,000 mg/day), there were no differences in recurrence rate (p = 0.38) and recurrence-free survival (p = 0.54) between the two groups.

Clinical Outcomes in the Metformin and Nonmetformin Subgroups among the Diabetic Patients with Cervical LN Metastasis of DTC

We performed a subgroup analysis of the 30 diabetic patients with cervical LN metastasis of DTC because LN metastasis was more prevalent in the metformin group than in the nonmetformin group. There were no significant differences in age, sex, BMI, HbA_{1c}, C-peptide, the surgeons, extent of surgery, tumor size, histology, T stage, extrathyroidal extension, radioiodine remnant ablation, or serum Tg at ablation between the metformin and nonmetformin subgroups (online suppl. table 1, see www.karger.com/doi/10.1159/000437365). Two of the 22 patients (9%) in the metformin subgroup and 3 of the 8 patients (38%) in the nonmetformin subgroup had recurrent DTC. The metformin subgroup had significantly longer DFS (17.1 ± 1.0 years) than the nonmetformin subgroup (8.6 ± 1.6 years; HR 0.16, 95% CI: 0.03–0.95, p = 0.021; fig. 1c), and metformin treatment was significantly associated with lower recurrence in multivariate analysis. The HR for metformin treatment was 0.02 (95% CI: 0.0004–0.85, p = 0.041) after adjustment for age, sex, BMI, and duration of diabetes, and 0.03 (95% CI: 0.002–0.47, p = 0.01) after adjustment for age, BMI, duration of diabetes, and extrathyroidal extension. This effect was also significant after adjusting for age, BMI, duration of diabetes, presence of tumor at resection margin, and serum Tg level at ablation (HR = 0.03, 95% CI: 0.001–0.78, p = 0.035; table 3). There was no significant difference in recurrence at distant organs between the metformin and nonmetformin subgroups (p = 0.47).

Discussion

In this study, we evaluated clinical outcomes in 60 diabetic patients and 210 nondiabetic patients matched for age, sex, BMI, and tumor size. There were no differences in the clinicopathological features of DTC in the two groups. We evaluated clinical outcomes in the diabetic patients according to metformin treatment and found that metformin was associated with a favorable outcome

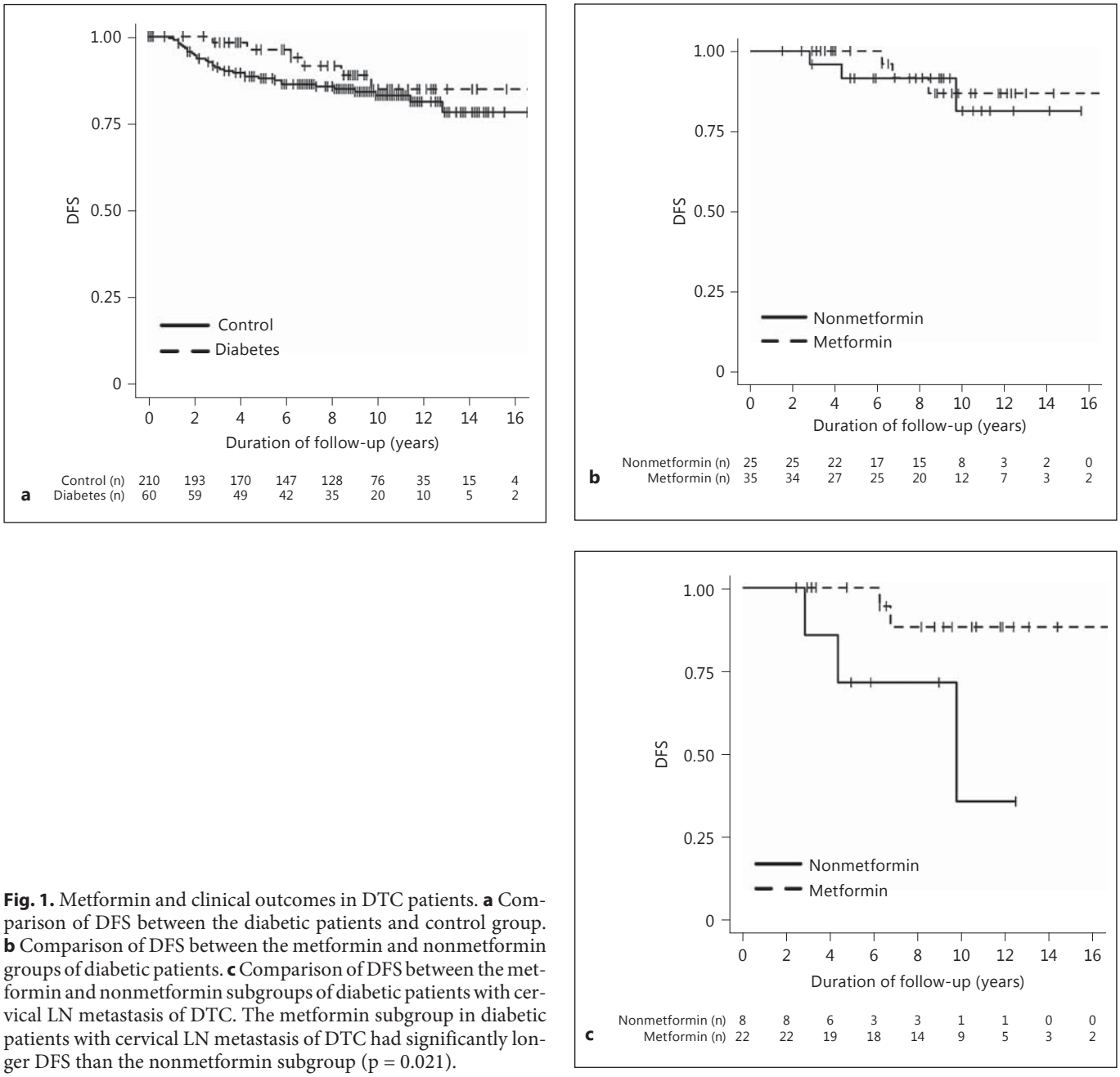

Fig. 1. Metformin and clinical outcomes in DTC patients. **a** Comparison of DFS between the diabetic patients and control group. **b** Comparison of DFS between the metformin and nonmetformin groups of diabetic patients. **c** Comparison of DFS between the metformin and nonmetformin subgroups of diabetic patients with cervical LN metastasis of DTC. The metformin subgroup in diabetic patients with cervical LN metastasis of DTC had significantly longer DFS than the nonmetformin subgroup ($p = 0.021$).

only in the diabetic patients with cervical LN metastasis of DTC: the metformin group had a significantly longer DFS than the nonmetformin group.

Previous studies have suggested that metformin has antineoplastic effects including induction of catabolism, downregulation of cell proliferation, cell cycle arrest, and apoptosis in the absence of tumor-specific compensatory mechanisms [1, 14]. Cancer cells replace the respiration of oxygen occurring in normal body cells by fermentation and produce large amounts of lactate by glycolysis even when oxygen supply is sufficient, so they require higher levels of mitochondrial oxidative phosphorylation. This fermentation phenomenon is called the 'Warburg effect' [15]. Previous work has shown that metformin inhibits oxidative phosphorylation and reduces ATP production [2, 16]. The decline in ATP level triggers activation of

Table 3. Cox proportional hazard models for cancer recurrence in diabetic DTC patients with cervical LN metastasis

Model	Variables	HR	95% CI	p
Model A	Age (years)	1.3	0.88–1.91	0.19
	Sex (male)	15.64	0.88–1.91	0.09
	BMI	1.42	0.82–2.45	0.21
	Duration of diabetes (years)	1.01	0.82–1.26	0.89
	Metformin (treatment)	0.02	0.0004–0.85	0.041
Model B	Age (years)	1.12	0.91–1.37	0.28
	BMI	1.52	0.83–2.78	0.17
	Duration of diabetes (years)	0.96	0.8–1.15	0.64
	ETE (present)	0.14	0.01–1.66	0.12
	Metformin (treatment)	0.03	0.002–0.47	0.01
Model C	Age (years)	0.74	0.86–2.1	0.19
	BMI	2.04	0.13–1.82	0.29
	Duration of diabetes (years)	1.51	0.42–1.04	0.07
	Tumor at resection margin (present)	0.66	0.11–21.55	0.75
	Tg at ablation (μg/l)	0.67	0.91–2.44	0.11
	Metformin (use)	0.03	0.001–0.78	0.035

Model A analyzed age at surgery, male sex, BMI at surgery, duration of diabetes, and metformin treatment. Model B analyzed age at surgery, BMI at surgery, duration of diabetes, presence of extrathyroidal extension, and metformin treatment. Model C analyzed age at surgery, BMI at surgery, duration of diabetes, presence of tumor at resection margin, serum Tg level at ablation, and metformin treatment. Age, BMI, duration of diabetes, and serum Tg level at ablation were included in the model as continuous variables. Duration of diabetes denotes time between diagnosis of diabetes and thyroid surgery. ETE = Extrathyroidal extension.

AMPK, which inhibits the mammalian target of rapamycin pathway and downregulates growth factor signaling [1, 2]. Nevertheless, cancer cells which are defective in AMPK signaling and downstream effectors are paradoxically hypersensitive to metformin. They cannot reduce their energy consumption by activating AMPK in conditions of energy stress, and therefore suffer an energy crisis and may undergo cell death [17, 18]. Metformin also leads to suppression of fatty acid synthase gene expression and inactivation of acetyl-CoA carboxylase. This causes reduction in lipogenesis and synthesis of the acetyl-CoA carboxylase product malonyl-CoA resulting in increased fatty acid oxidation. Moreover, AMPK activation may result in *p53* activation, which is a tumor suppressor that is often mutated in other type of cancer [19, 20]. There have been several in vitro and in vivo studies on papillary, medullary, and anaplastic thyroid cancer cell lines evaluating the effects of metformin on the suppression of clonal growth, self-renewal, and proliferation of cancer stem cells [9, 10, 21, 22].

Several clinical studies have suggested that metformin is beneficial in diabetic patients in preventing the progression of certain cancers [1, 3–5, 7, 8, 23]. Cancer patients treated with metformin before cancer diagnosis had significantly increased survival than nondiabetic patients or diabetics taking other antidiabetic medications [3, 7, 8, 24]. Some studies have suggested that increased serum insulin was associated with cancer proliferation, and both insulin levels and cancer cell proliferation were blunted by metformin [7, 25]. Although Klubo-Gwiezdzinska et al. [9] reported that metformin was an independent factor increasing the remission rate and progression-free survival of DTC patients with diabetes, there have been few studies of the association between metformin and the prognosis of thyroid cancer.

It is difficult to establish an effect of metformin on the prognosis of DTC because many clinical factors such as BMI, insulin resistance, cancer subtype, and drug level can influence its effects [26–28]. It has been reported that metformin caused a marked reduction of Ki-67 in a subgroup of breast cancer patients with high BMI, high insulin resistance, or high C-reactive protein levels [26]. In our study, metformin treatment was associated with a favorable outcome only in diabetic patients with cervical

LN metastasis of DTC even though cervical LN metastasis was more prevalent in the metformin group than the non-metformin group. We found that metformin was associated with prolonged DFS in these patients at high risk for DTC recurrence.

This study had the limitation that it was retrospective and there was only a small number of diabetic patients with DTC. We matched for age, sex, BMI, and tumor size to avoid the selection bias. However, we did not control the pathological characteristics of DTC except the tumor size because only tumor size could be preoperatively estimated. It was an incidental finding that cervical LN metastasis was more prevalent in the metformin group than control group. In this study, thyroid surgery was done by four surgeons. The recurrence rate could be affected by the extent of surgery and the skills of the surgeons. However, there were no significant differences in the surgeons and extent of surgery between the metformin and non-metformin groups. Nevertheless, this is the first study to suggest that the metformin effect is only significant in the advanced stage of DTC. A further larger study is needed to establish a clear protective effect of metformin on DTC recurrence.

In conclusion, metformin treatment is associated with longer DFS in diabetic patients with cervical LN metastasis of DTC. Our findings suggest that metformin has an antineoplastic effect at a certain stage of DTC.

Acknowledgements

This study was supported by the National Research Foundation of Korea (DIRAMS) grant funded by the Korea government (MSIP, No. 50600-2014).

Disclosure Statement

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

References

1 Pierotti MA, Berrino F, Gariboldi M, Melani C, Mogavero A, Negri T, Pasanisi P, Pilotti S: Targeting metabolism for cancer treatment and prevention: metformin, an old drug with multi-faceted effects. Oncogene 2013;32:1475–1487.

2 Pollak MN: Investigating metformin for cancer prevention and treatment: the end of the beginning. Cancer Discov 2012;2:778–790.

3 Jiralerspong S, Palla SL, Giordano SH, Meric-Bernstam F, Liedtke C, Barnett CM, Hsu L, Hung MC, Hortobagyi GN, Gonzalez-Angulo AM: Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. J Clin Oncol 2009;27:3297–3302.

4 Lee MS, Hsu CC, Wahlqvist ML, Tsai HN, Chang YH, Huang YC: Type 2 diabetes increases and metformin reduces total, colorectal, liver and pancreatic cancer incidences in Taiwanese: a representative population prospective cohort study of 800,000 individuals. BMC Cancer 2011;11:20.

5 Bo S, Ciccone G, Rosato R, Villois P, Appendino G, Ghigo E, Grassi G: Cancer mortality reduction and metformin: a retrospective cohort study in type 2 diabetic patients. Diabetes Obes Metab 2012;14:23–29.

6 Rieken M, Xylinas E, Kluth L, Trinh QD, Lee RK, Fajkovic H, Novara G, Margulis V, Lotan Y, Martinez-Salamanca JI, Matsumoto K, Seitz C, Remzi M, Karakiewicz PI, Scherr DS, Briganti A, Kautzky-Willer A, Bachmann A, Shariat SF: Diabetes mellitus without metformin intake is associated with worse oncologic outcomes after radical nephroureterectomy for upper tract urothelial carcinoma. Eur J Surg Oncol 2014;40:113–120.

7 Hou G, Zhang S, Zhang X, Wang P, Hao X, Zhang J: Clinical pathological characteristics and prognostic analysis of 1,013 breast cancer patients with diabetes. Breast Cancer Res Treat 2013;137:807–816.

8 Garrett CR, Hassabo HM, Bhadkamkar NA, Wen S, Baladandayuthapani V, Kee BK, Eng C, Hassan MM: Survival advantage observed with the use of metformin in patients with type II diabetes and colorectal cancer. Br J Cancer 2012;106:1374–1378.

9 Klubo-Gwiezdzinska J, Costello J Jr, Patel A, Bauer A, Jensen K, Mete M, Burman KD, Wartofsky L, Vasko V: Treatment with metformin is associated with higher remission rate in diabetic patients with thyroid cancer. J Clin Endocrinol Metab 2013;98:3269–3279.

10 Chen G, Xu S, Renko K, Derwahl M: Metformin inhibits growth of thyroid carcinoma cells, suppresses self-renewal of derived cancer stem cells, and potentiates the effect of chemotherapeutic agents. J Clin Endocrinol Metab 2012;97:E510–E520.

11 Mazzaferri EL: An overview of the management of papillary and follicular thyroid carcinoma. Thyroid 1999;9:421–427.

12 Eustatia-Rutten CF, Corssmit EP, Biermasz NR, Pereira AM, Romijn JA, Smit JW: Survival and death causes in differentiated thyroid carcinoma. J Clin Endocrinol Metab 2006;91:313–319.

13 Yim JH, Kim EY, Bae Kim W, Kim WG, Kim TY, Ryu JS, Gong G, Hong SJ, Yoon JH, Shong YK: Long-term consequence of elevated thyroglobulin in differentiated thyroid cancer. Thyroid 2013;23:58–63.

14 Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F: Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond) 2012;122:253–270.

15 Warburg O: On the origin of cancer cells. Science 1956;123:309–314.

16 Hardie DG, Ross FA, Hawley SA: AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nat Rev Mol Cell Biol 2012;13:251–262.

17 Buzzai M, Jones RG, Amaravadi RK, Lum JJ, DeBerardinis RJ, Zhao F, Viollet B, Thompson CB: Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. Cancer Res 2007;67:6745–6752.

18 Algire C, Amrein L, Bazile M, David S, Zakikhani M, Pollak M: Diet and tumor LKB1 expression interact to determine sensitivity to anti-neoplastic effects of metformin in vivo. Oncogene 2011;30:1174–1182.

19 Micic D, Cvijovic G, Trajkovic V, Duntas LH, Polovina S: Metformin: its emerging role in oncology. Hormones (Athens) 2011;10:5–15.

20 Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE: Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 2001;108:1167–1174.

21 Moon HS, Mantzoros CS: Regulation of cell proliferation and malignant potential by irisin in endometrial, colon, thyroid and esophageal cancer cell lines. Metabolism 2014;63:188–193.
22 Pappa T, Alevizaki M: Metformin and thyroid: an update. Eur Thyroid J 2013;2:22–28.
23 Skinner HD, McCurdy MR, Echeverria AE, Lin SH, Welsh JW, O'Reilly MS, Hofstetter WL, Ajani JA, Komaki R, Cox JD, Sandulache VC, Myers JN, Guerrero TM: Metformin use and improved response to therapy in esophageal adenocarcinoma. Acta Oncol 2013;52:1002–1009.
24 Currie CJ, Poole CD, Jenkins-Jones S, Gale EA, Johnson JA, Morgan CL: Mortality after incident cancer in people with and without type 2 diabetes: impact of metformin on survival. Diabetes Care 2012;35:299–304.
25 Hadad S, Iwamoto T, Jordan L, Purdie C, Bray S, Baker L, Jellema G, Deharo S, Hardie DG, Pusztai L, Moulder-Thompson S, Dewar JA, Thompson AM: Evidence for biological effects of metformin in operable breast cancer: a pre-operative, window-of-opportunity, randomized trial. Breast Cancer Res Treat 2011;128:783–794.
26 Bonanni B, Puntoni M, Cazzaniga M, Pruneri G, Serrano D, Guerrieri-Gonzaga A, Gennari A, Trabacca MS, Galimberti V, Veronesi P, Johansson H, Aristarco V, Bassi F, Luini A, Lazzeroni M, Varricchio C, Viale G, Bruzzi P, Decensi A: Dual effect of metformin on breast cancer proliferation in a randomized presurgical trial. J Clin Oncol 2012;30:2593–2600.
27 DeCensi A, Puntoni M, Gandini S, Guerrieri-Gonzaga A, Johansson HA, Cazzaniga M, Pruneri G, Serrano D, Schwab M, Hofmann U, Mora S, Aristarco V, Macis D, Bassi F, Luini A, Lazzeroni M, Bonanni B, Pollak MN: Differential effects of metformin on breast cancer proliferation according to markers of insulin resistance and tumor subtype in a randomized presurgical trial. Breast Cancer Res Treat 2014;148:81–90.
28 Niraula S, Dowling RJ, Ennis M, Chang MC, Done SJ, Hood N, Escallon J, Leong WL, McCready DR, Reedijk M, Stambolic V, Goodwin PJ: Metformin in early breast cancer: a prospective window of opportunity neoadjuvant study. Breast Cancer Res Treat 2012;135:821–830.