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Oxidative Stress-regulating Enzymes and Endometrial Cancer Survival in Relation to Metformin Intake in Diabetic Patients

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Abstract. *Background/Aim: Metformin inhibits tumorigenesis in endometrial carcinoma and interferes with the expression of oxidative stress-regulating proteins, such as nuclear factor erythroid 2-related factor 2 (Nrf2) and Kelch-like ECH-associated protein 1 (Keap1). Although manganese superoxide dismutase (MnSOD) is vital for withstanding mitochondrial oxidative stress, it has also been linked with chemoresistance and poorer outcomes in several cancer types. However, data on endometrial cancers are limited. This study aimed to highlight the relationship between mitochondrial redox regulation and endometrial cancer survival in relation to metformin consumption in women with type 2 diabetes mellitus (T2DM). Patients and Methods: Our retrospective hospital-based cohort study included 121 patients diagnosed with endometrial carcinoma and T2DM between 2007 and 2014. Fifty-eight patients were using metformin at the time of diagnosis. Nrf2 and Keap1 expression levels in the tumor samples were assessed immunohistochemically, and MnSOD levels were measured both immunohistochemically and from the serum samples. Results: High MnSOD tissue expression was associated with better overall survival among metformin users in the univariate analysis (p=0.03). When adjusted for histology and stage, high serum MnSOD was associated with better overall survival (HR=0.22, 95%CI=0.07-0.71, p=0.01). No association was found between MnSOD, Nrf2, or Keap1 and overall survival among metformin non-users. Conclusion: Higher expression of MnSOD in patients with endometrial cancer and T2DM is associated with better overall survival if the patient is consuming metformin.*

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Key Words: Endometrial carcinoma, metformin, type 2 diabetes, oxidative stress, Nrf2, Keap1, MnSOD.

Endometrial cancer is the sixth most common cancer type among women worldwide (1), and incidence rates have increased in recent decades (2). Type 2 diabetes mellitus (T2DM)-associated hyperglycemia and chronic inflammation create a favorable environment for tumorigenesis (3). The incidence of T2DM, which has been implicated in the development of numerous malignancies, such as endometrial cancer, is rapidly increasing worldwide (3). The frequently used antidiabetic medication (ADM) metformin has antiproliferative, anti-invasive, and antimetastatic properties *in vitro* (4). Metformin is also associated with a better outcome in patients with endometrial cancer and T2DM (3-10).

Although reactive oxygen species (ROS) are continuously produced in all cell types, for instance, as a by-product of the mitochondrial respiratory chain, their excessive production is detrimental (11). Thus, cells are equipped with an array of protective mechanisms to prevent oxidative stress (11). Nuclear factor erythroid 2-related factor 2 (Nrf2) plays a key role in regulating cellular responses to oxidative stress (12). Nrf2 activates antioxidant response element genes that control antioxidant production, autophagy, inflammation, apoptosis, and mitochondrial functions (11). During the usual homeostatic state, Nrf2 is ubiquitinated by the Kelch-like ECH-associated protein

1 (Keap1) and then transferred to the proteasome for degradation (11, 12). The presence of ROS prevents Nrf2/Keap1 interaction; therefore, Nrf2 is active only under oxidative stress (12). The protective function of Nrf2 can be exploited by tumor cells (11). High levels of Nrf2 protect against oxidative damage and promote chemoresistance (13). Increased Nrf2 expression is associated with lymph node metastasis and poorer prognosis in type II endometrial carcinoma (13, 14).

The role of mitochondrial ROS in tumorigenesis has long been established (15). Manganese superoxide dismutase (MnSOD) is a mitochondrial antioxidant enzyme that ensures mitochondrial function during oxidative stress and has a crucial antiapoptotic role (16). The atypical expression of MnSOD affects various stages and characteristics of cancer (16). Low MnSOD expression during the early stages of carcinogenesis promotes tumor growth (16), whereas high MnSOD expression is associated with chemoresistance and a worse prognosis as cancer progresses (17).

The use of metformin has been observed to inhibit Nrf2 expression independently of Keap1 by blocking other pathways involved in endometrial cancer chemoresistance (18-20). Furthermore, high levels of Nrf2 predict poorer outcomes among diabetic breast cancer patients who do not use metformin compared with those who do (8). In addition, *in vitro*, metformin has been shown to decrease cellular ROS levels while simultaneously increasing the expression of MnSOD (21).

This retrospective study aimed to further explore the relationship between oxidative stress and endometrial cancer survival in patients with T2DM. We investigated the expression of critical redox-state regulators in endometrial cancer tissue and serum of metformin users and non-users, focusing on the Nrf2/Keap1 axis and the function of MnSOD.

Patients and Methods

Patients. Our study cohort consisted of 121 patients with T2DM who were diagnosed with endometrial cancer at Oulu University Hospital in Finland between 2007 and 2014. Written informed consent for participation was obtained during the first hospital visit. Data were gathered from hospital records. The collected data included each patient's age, parity, antidiabetic medication, menopause age, body mass index (BMI), and the presence of fatty liver disease along with cancer-related information, such as stage, histology, peritoneal cytology, lymphovascular invasion, estrogen receptor status, adjuvant treatment, and the presence of residual tumors after surgery.

The patients were classified as either metformin users or non-users according to the ADM used at the time of endometrial cancer diagnosis. The patients were categorized as metformin users if they had used metformin alone or in combination with other oral ADMs. If the patients consumed only other forms of oral ADMs, insulin (alone or combined with metformin and/or other oral ADMs), or did not use any ADMs, they were categorized as metformin non-users.

All endometrial cancer diagnoses were based on histology, and the stages were re-checked and reported according to the current International Federation of Gynecology and Obstetrics (FIGO) classification by a gynecological oncologist (22). FIGO stages I A and I B were categorized as early stage, whereas the advanced stage included stages II, III, and IV. Endometrial cancers were labeled as type 1 and type 2 cancers according to this histology. Type 1 cancers included grades 1 and 2 endometrioid cancers and mucinous cancers. Type 2 cancers included grade 3 endometrioid, serous, clear cell, mixed, and undifferentiated endometrial cancers along with carcinosarcomas.

The follow-up period began at the time of endometrial cancer surgery, except in cases ineligible for surgery (11.6%). In these cases, the follow-up period started at the time of endometrial cancer diagnosis. The follow-up period ended with the death of the patient or the cessation of the follow-up period (August 7, 2018).

Immunohistochemistry. Keap1, Nrf2, and MnSOD expression was assessed by immunohistochemistry (IHC). The samples of tumor tissues were collected during primary surgery. Diagnostic endometrial biopsy was used in cases ineligible for surgery. Tissue sections measuring 3.5 μm were cut from a representative paraffin block and placed on SuperFrostPlus glass slides (Menzel-gläser, Braunschweig, Germany). These sections were first de-paraffinized in xylene, rehydrated in a descending series of ethanol concentrations, and then rinsed. Antigen retrieval was completed in 10 mM citrate buffer (pH 6.0) in a microwave oven for 4 min followed by cooling at room temperature. The slides were immersed in 3% hydrogen peroxide and methanol for 15 min so the endogenous peroxide could be consumed.

Immunostainings were carried out using rabbit monoclonal anti-Nrf2 (EPI 808Y, Abcam, Cambridge, UK) at 1:300 dilution, goat polyclonal anti-Keap1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1:100 dilution, and rabbit anti-MnSOD (Sigma-Aldrich, St Louis, MO, USA) at 1:1,000 dilution. The Dako Envision Kit (Dako, Glostrup, Denmark) was used to detect Nrf2, the goat-on-rodent HRP-polymer kit (Biocare GHP516L, Biocare Medical, Concord, CA, USA) was used to detect Keap1, and the Novolink Polymer Detection System (Leica Biosystems, Newcastle Ltd, Newcastle upon Tyne, UK) was used to detect MnSOD. Aminoethyl carbazole (Zymed Laboratories Inc., San Francisco, CA, USA) was used as a chromogen. Meyer's hematoxylin immersed in 2% ammonia water was used for counterstaining, after which the sections were mounted with Immu-Mount (Shandon, Pittsburgh, PA, USA). Negative controls were prepared using the same procedure with the exception of primary antibodies, which were replaced with PBS or serum isotype controls (Zymed Laboratories Inc.).

Immunoreactivity in the samples was assessed by the intensity of staining in the cytoplasm of the tumor cells and by the proportion of positively stained tumor cells. Immunoreactivity was also evaluated by two independent investigators (E.U., A.A.) who were blinded to the clinical data. The staining reactions were categorized into four groups: 0 for no staining intensity and no positive or only a few positive cells; 1 for weak staining intensity (>20-49% of positive cells); 2 for moderate staining intensity (>50-89% of positive cells); and 3 for strong staining intensity (>90% of positive cells). If the results of the immunoreactivity evaluation differed between the investigators, the sample was re-evaluated until a consensus was reached. For the statistical analysis, the immunoreactivity results were separated into two groups: low expression (0-1) and high expression (2-3). Examples of Nrf2, Keap1 and MnSOD expression assessed by immunohistochemistry are shown in Figure 1.

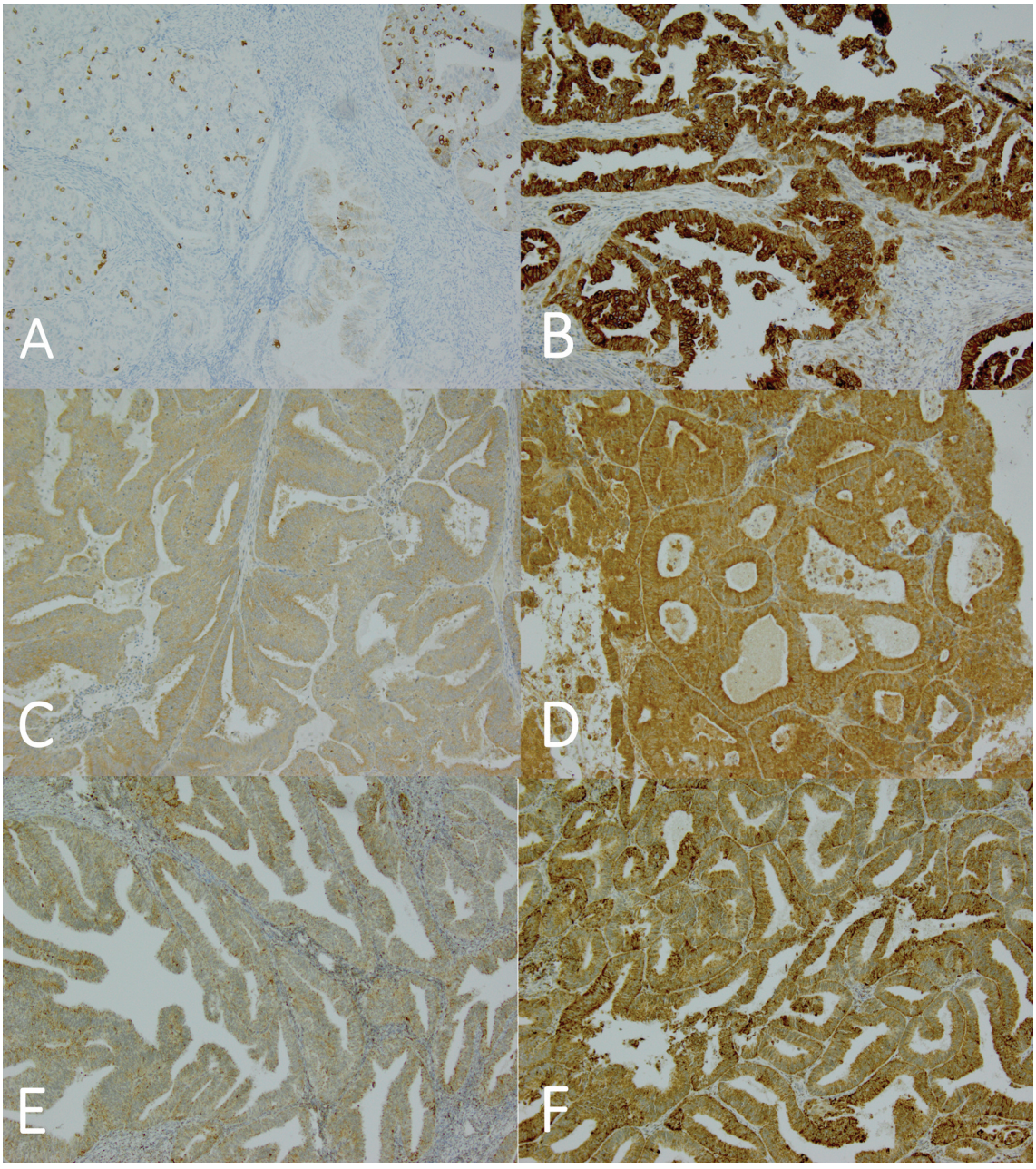

Figure 1. *Nuclear factor erythroid 2-related factor 2 (Nrf2), Kelch-like ECH-associated protein 1 (Keap1) and manganese superoxide dismutase (MnSOD) expression assessed by immunohistochemistry (IHC), magnification ×100. A) Low Nrf2 expression (graded as 1), B) high Nrf2 expression (graded as 3), C) low Keap1 expression (graded as 1), D) high Keap1 expression (graded as 3), E) low MnSOD expression (graded as 1), and F) high MnSOD expression (graded as 3).*

Serum samples. Serum samples were collected during the first hospital visit. The serum samples were stored at –70˚C until analyzed. MnSOD levels were measured with the commercial LF-EK0104 ELISA kit (LF-EK0104, AbFrontier, Seoul, Republic of Korea) following the manufacturer's protocols. The median level of serum MnSOD was used to categorize the results into two groups: low and high.

Statistical analysis. The IBM SPSS Statistics software, version 28.0.1.0 (IBM Corporation, Armonk, NY, USA) was used to perform the statistical analysis, whereas the GraphPad Prism software version 8.0.2 (GraphPad Software, San Diego, CA, USA) was used to draw the Kaplan–Meier curves. The R environment version 4.3.1. was used to draw Figure 2 of the enzyme expression distribution. Continuous variables between high and low protein

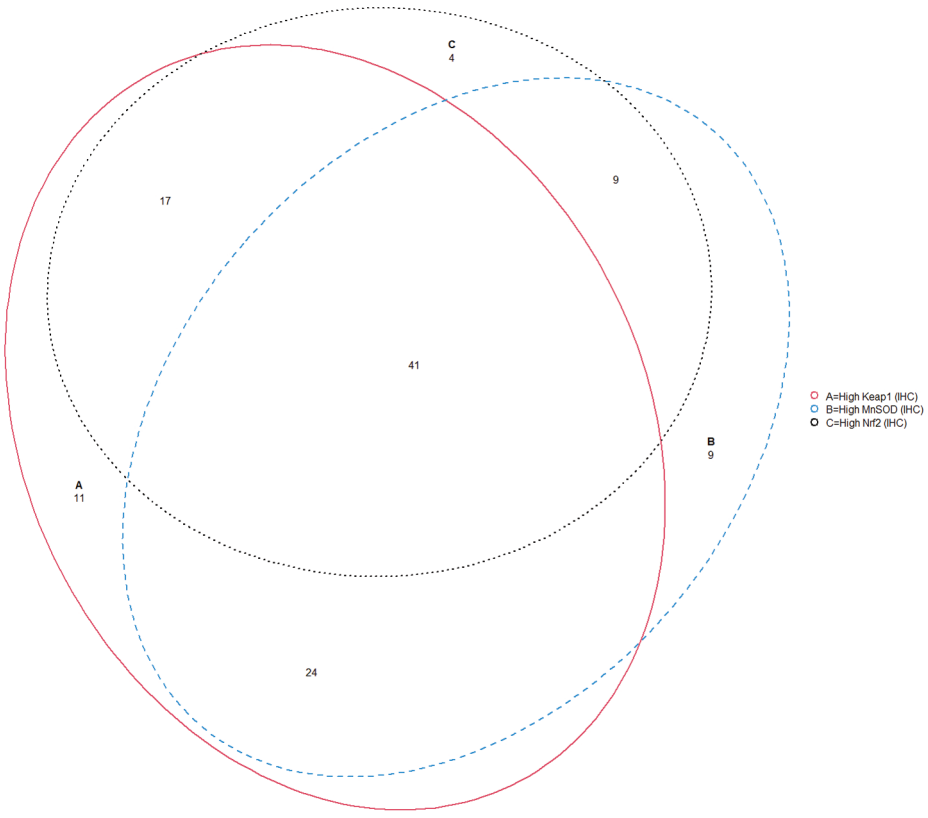

Figure 2. *Distribution of high enzyme expression in the study cohort. IHC: Immunohistochemistry; Nrf2: nuclear factor erythroid 2-related factor 2; Keap1: Kelch-like ECH-associated protein 1; MnSOD: manganese superoxide dismutase.*

expression groups were analyzed using the two-sample *t*-test and Mann–Whitney *U*-test. Pearson's chi-squared test or Fisher's exact test was used to assess categorical variables between the expression groups. The overall survival (OS) was evaluated with the Kaplan–Meier log-rank test. In the multivariate analysis, histology, stage, and protein expression were included in the model. All statistical analyses were performed independently in the metformin user and metformin non-user groups. A *p*-value of 0.05 or less was considered statistically significant.

Results

Patient characteristics. Our study population of 121 patients was divided into two groups based on their use of metformin: 47.9% (n=58) of the patients were included in the metformin user group, while the remaining 52.1% (n=63) were included in the metformin non-user group (Table I). Patient age at diagnosis varied from 51 to 88 years old. The mean age was 70.5 years in the metformin user group and 71.2 in the metformin non-user group. Obesity was somewhat more common in patients who were metformin non-users. The median BMI was 33.0 kg/m^2 among metformin users and 36.0 kg/m^2 among metformin non-users (data not shown). The two medication groups differed in terms of tumor characteristics. In metformin users, type 2 histology, advanced stage, and lymphovascular invasion were more prominent characteristics compared with the metformin non-user group; these findings were reported in a previous publication (23).

Table I. *Distribution of antidiabetic medication (ADM).*

Metformin users (n=58)	Only metformin (n=35)
	Metformin + other oral ADM (n=23)
Metformin non-users (n=63)	Only insulin (n=12)
	Only other oral ADM (n=8)
	Insulin + metformin (n=14)
	Insulin + other oral ADM (n=3)
	Insulin + metformin + other oral ADM (n=8)
	No ADM (n=18)

Enzyme expression and serum levels. In the metformin user group, 41.4% (n=24) of the patients expressed low levels of

Table II. *Enzyme expression according to patient characteristics in metformin users.*

	Nrf2 expression (IHC)			Keap1 expression (IHC)			MnSOD expression (IHC)			MnSOD (serum levels)*		
	Low (N=24)	High (N=34)	*p*-Value	Low (N=11)	High (N=47)	*p*-Value	Low (N=19)	High (N=39)	*p*-Value	Low (N=18)	High (N=26)	*p*-Value
Age (years)												
Mean	70.5	70.5	0.99[a]	70.5	70.5	0.98[a]	69.9	70.8	0.70[a]	72.0	67.9	0.13[a]
Range	58-88	51-84		60-79	51-88		59-81	51-88		60-88	51-84	
BMI (kg/m^2)**												
Median	32	34	0.08[b]	34	33	0.90[b]	32	34	0.16[b]	33.5	32	0.45[b]
Range	19-48	23-55		25-41	19-55		23-55	19-51		23-55	22-48	
Menopause age			_ [d]			_ [d]			_ [d]			_ [d]
Premenopausal	1	0		0	1		1	0		0	0	
<50	3	3		1	5		2	4		2	2	
50-53	8	16		5	19		10	14		11	9	
≥54	8	11		4	14		5	13		3	11	
Parity												
Median	2	2.5	0.92[b]	2	3	0.26[b]	2	2	0.41[b]	2.5	2	0.31[b]
Range	0-6	0-9		0-9	0-8		0-9	0-8		0-9	0-5	
Adjuvant treatment			_ [d]			_ [d]			_ [d]			_ [d]
None	11	16		6	21		8	19		9	14	
WPRT	7	8		2	13		4	11		3	7	
Chemotherapy	6	7		1	12		6	7		3	8	
Vaginal brachytherapy	5	6		3	8		5	6		6	2	
Intracavitary radiation	0	2		0	2		0	2		0	0	
Hormonal treatment	0	1		0	1		0	1		0	0	

[a]*t*-test, [b]Mann-Whitney, [d]Comparison not relevant. *Data missing from 14 patients. **Data missing from 4 patients. IHC: Immunohistochemistry; Nrf2: nuclear factor erythroid 2-related factor 2; Keap1: kelch-like ECH-associated protein 1; MnSOD: manganese superoxide dismutase; BMI: body mass index; WPRT: whole pelvic radiotherapy.

Nrf2, whereas 58.6% (n=34) expressed high levels. Keap1 expression was low in 19.0% (n=11) of the patients and high in 81.0% (n=47). In the same ADM group, 32.8% (n=19) of the metformin users expressed low levels of MnSOD, whereas 67.2% (n=39) expressed high levels. Serum MnSOD expression was low in 31.0% (n=18) and high in 44.8% (n=26) of the patients. Figure 1 shows examples of the immunohistochemical staining and protein expression. When assessed immunohistochemically, a high expression of one enzyme was likely paired with a high expression of another or all three enzymes, regardless of the ADM group (Figure 2).

Neither Nrf2 nor Keap1 expression levels showed an association with age, BMI, histology, stage, myometrial invasion, lymphovascular invasion, or estrogen receptor status in the metformin user group (Table II and Table III). However, Keap1 expression demonstrated an association with estrogen receptor status (p=0.01) among metformin non-users (data not shown). MnSOD expression or serum levels exhibited no association with tumor or patient characteristics in either ADM group.

Survival. In the univariate analysis, high immunohistochemical MnSOD expression was associated with better OS in the metformin user group (p=0.03, Figure 3), which was not observed in the metformin non-users (p=0.60, data not shown). Nrf2, Keap1, and serum MnSOD expression did not have a notable effect on OS in the univariate analysis.

We performed four Cox regression analyses, each containing stage, histology, and one of the studied proteins. In all of these analyses, type 2 histology was the most dominant indicator of poor prognosis in the OS. Intriguingly, the advanced stage was not statistically significantly associated with a worse prognosis. High serum MnSOD was associated with better OS [hazard ratio (HR)=0.22, 95% confidence interval (CI)=0.07-0.71] in metformin users (Table IV). Nrf2 (HR=0.64, 95%CI=0.23-1.61), Keap1 (HR=1.32, 95%CI=0.36-4.90), and MnSOD (HR=0.40, 95%CI=0.15-1.07) tissue expression was not associated with OS.

Discussion

The present study was the first to highlight the relationship between redox-regulating enzyme expression and endometrial cancer survival in relation to metformin usage. As the main finding, strong MnSOD tumor tissue expression and high MnSOD serum levels were associated with better OS in metformin users but not in metformin non-users.

Table III. *Enzyme expression according to tumor characteristics in metformin users.*

	Nrf2 expression (IHC)			Keap1 expression (IHC)			MnSOD expression (IHC)			MnSOD (serum levels)*		
	Low (N=24)	High (N=34)	*p*-Value	Low (N=11)	High (N=47)	*p*-Value	Low (N=19)	High (N=39)	*p*-Value	Low (N=18)	High (N=26)	*p*-Value
Histology												
Type 1	16 (42.1%)	22 (57.9%)	– [d]	6 (15.8%)	32 (84.2%)	0.49[c]	10 (26.3%)	28 (73.7%)	0.24[c]	12 (46.2%)	14 (53.8%)	0.54[c]
Type 2	8 (40.0%)	12 (60.0%)		5 (25.0%)	15 (75.0%)		9 (45.0%)	11 (55.0%)		6 (33.3%)	12 (66.0%)	
Stage												
Early	15 (40.5%)	22 (59.5%)	0.78[c]	8 (21.6%)	29 (78.4%)	0.73[c]	11 (29.7%)	26 (70.3%)	0.76[c]	11 (39.3%)	17 (60.7%)	– [d]
Advanced	9 (47.4%)	10 (52.6%)		3 (15.8%)	16 (84.2%)		7 (36.8%)	12 (63.2%)		7 (43.8%)	9 (56.2%)	
Unknown	2			2			2			14		
Deep myometrial invasion												
Yes	13 (50.0%)	13 (50.0%)	0.58[c]	6 (23.1%)	20 (76.9%)	0.74[c]	9 (34.6%)	17 (65.4%)	– [d]	9 (45.0%)	11 (55.0%)	0.76[c]
No	11 (39.3%)	17 (60.7%)		5 (17.9%)	23 (82.1%)		9 (32.1%)	19 (67.9%)		9 (37.5%)	15 (62.5%)	
Unknown	4			4			4			14		
Lymphovascular invasion												
Yes	11 (45.8%)	13 (54.2%)	0.79[c]	5 (20.8%)	19 (79.2%)	– [d]	10 (41.7%)	14 (58.3%)	0.40[c]	8 (40.0%)	12 (60.0%)	– [d]
No	13 (41.9%)	18 (58.1%)		6 (19.4%)	25 (80.6%)		9 (29.0%)	22 (71.0%)		10 (41.7%)	14 (58.3%)	
Missing	3			3			3			14		
ER status												
Positive	20 (40.8%)	29 (59.2%)	0.69[c]	9 (18.4%)	40 (81.6%)	0.61[c]	15 (30.6%)	34 (69.4%)	– [d]	16 (42.1%)	22 (57.9%)	– [d]
Negative	2 (28.6%)	5 (71.4%)		2 (28.6%)	5 (71.4%)		2 (28.6%)	5 (71.4%)		2 (40.0%)	3 (60.0%)	
Unknown	2			2			2			15		
Residual tumor after surgery			– [d]			– [d]			– [d]			– [d]
No	22	27		10	39		15	34		17	23	
Yes	1	3		1	3		2	2		1	3	
No surgery	1	4		0	5		2	3		0	0	

[c]Pearson's chi square, [d]Comparison not relevant. *Data missing from 14 patients. IHC: Immunohistochemistry; Nrf2: nuclear factor erythroid 2-related factor 2; Keap1: Kelch-like ECH-associated protein 1; MnSOD: manganese superoxide dismutase; ER: estrogen receptor.

Table IV. *Overall survival in metformin users and metformin non-users according to the Cox regression model.*

	Metformin users			Metformin non-users		
	Hazard ratio	95% Confidence interval	*p*-Value	Hazard ratio	95% Confidence interval	*p*-Value
Histology (type 1 *vs.* type 2)	5.39	1.68-17.28	<0.01	3.42	1.11-10.54	0.03
Stage (early *vs.* advanced)	2.70	0.85-8.53	0.09	3.00	0.98-9.18	0.05
Nrf2 (IHC) (low *vs.* high)	0.64	0.23-1.61	0.34	1.71	0.56-5.21	0.34
Histology	5.68	1.67-19.31	0.05	3.00	0.98-9.11	0.05
Stage	2.44	0.72-8.26	0.15	3.38	1.10-10.44	0.03
Keap1 (IHC)	1.32	0.36-4.90	0.68	0.73	0.24-2.18	0.57
Histology	5.26	1.52-18.19	<0.01	2.97	0.98-8.97	0.05
Stage	2.40	0.71-8.16	0.16	3.20	1.06-9.69	0.04
MnSOD (IHC)	0.40	0.15-1.07	0.07	1.90	0.42-8.48	0.40
Histology	15.13	2.69-85.28	<0.01	3.44	0.92-12.96	0.07
Stage	3.04	0.72-12.73	0.13	2.20	0.52-9.23	0.28
MnSOD (serum)	0.22	0.07-0.71	0.01	1.21	0.32-4.66	0.78

IHC: Immunohistochemistry; Nrf2: nuclear factor erythroid 2-related factor 2; Keap1: Kelch-like ECH-associated protein 1; MnSOD: manganese superoxide dismutase.

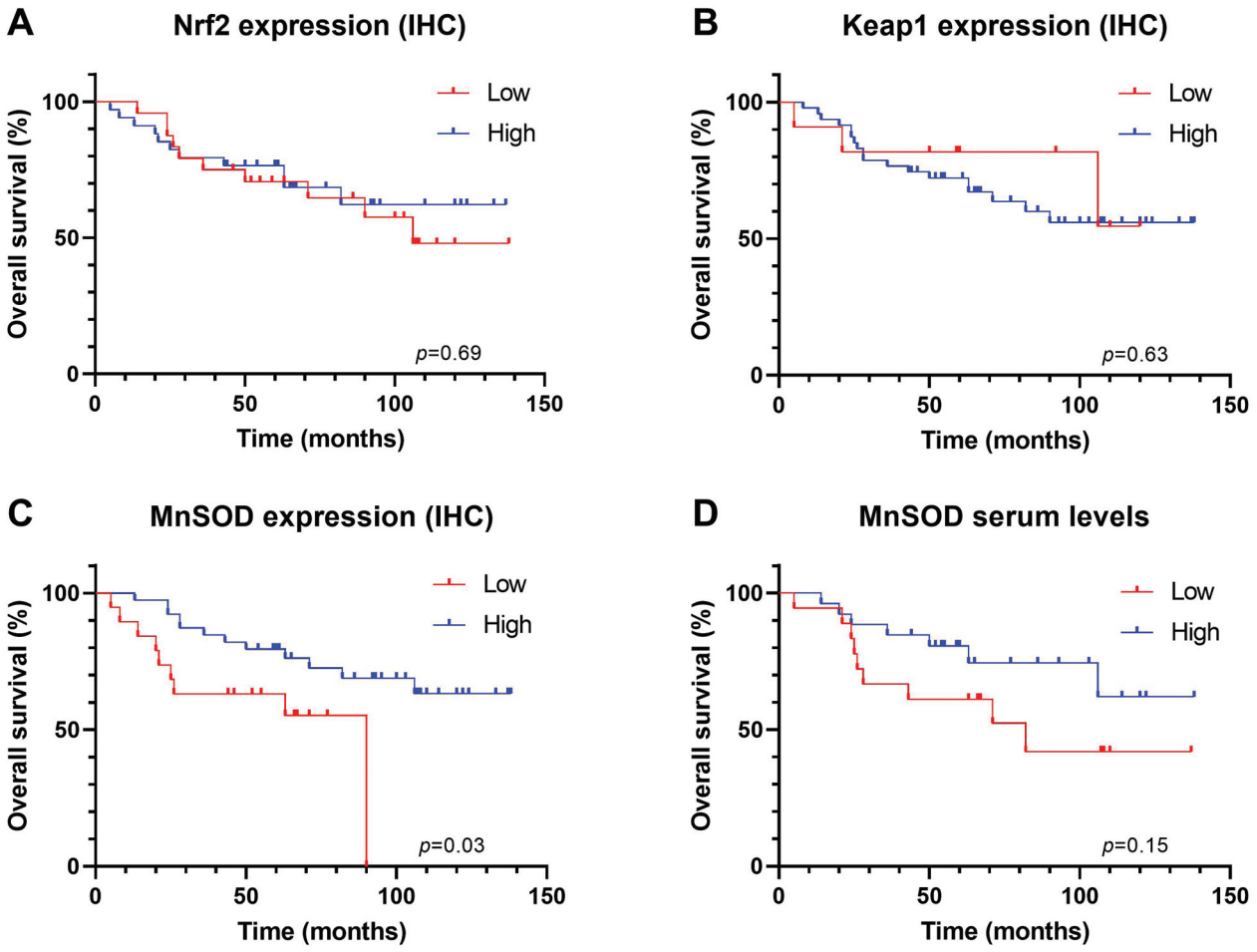

Figure 3. *Kaplan–Meier graphs in metformin users. IHC: Immunohistochemistry; Nrf2: nuclear factor erythroid 2-related factor 2; Keap1: Kelch-like ECH-associated protein 1; MnSOD: manganese superoxide dismutase.*

Prior studies have established high MnSOD expression as a negative prognostic factor in other cancer types (15-17, 24). However, the effect of metformin consumption has not yet been added to the equation. Becuwe *et al*. (2014) found that altered MnSOD expression was associated with poor prognostic factors in advanced breast cancer (16). Similar findings were presented by Fu *et al*. (2016), who stated that MnSOD enhances chemoresistance and metastatic potential in circulating breast cancer cells in animal models (24). Additionally, Amano *et al*. (2019) demonstrated that immunohistochemically measured strong MnSOD expression is associated with chemoresistance and poor prognosis in ovarian cancer (17). In their review, Idelchik *et al*. (2018) specified that MnSOD both inhibits tumorigenesis during the initial stages of cancer and promotes tumor growth and metastatic potential during the more advanced stages (15).

MnSOD has traditionally been associated with poorer prognostic factors. Contrary to most previous studies, our findings suggest that high serum MnSOD expression may be connected to prolonged overall survival in metformin users, although further studies are needed to verify this link. Sharma and Kumar (2018) found that metformin decreases ROS levels while promoting the expression of SOD enzymes in breast cancer cell lines (21). Tumor cells treated with metformin were shown to undergo apoptosis via mitochondrial dysfunction (21). These results indicate a close interplay between metformin and SOD enzymes.

The role of metformin in endometrial cancer has been previously studied; however, the data have remained somewhat inconclusive. While metformin usage may have favorable effects on the prognosis of endometrial cancer, studies that have observed these effects are heterogeneous; hence, conclusions cannot be drawn (7). Studies on the relationship between metformin and oxidative stress in endometrial cancer are limited. Metformin has been shown to alter the Nrf2/Keap1 axis, and high Nrf2 expression has been found to predict poorer

outcomes in patients with breast cancer who do not consume metformin compared with those who do (8).

The exact intracellular mechanisms responsible for the impact of metformin on redox-regulating enzymes have previously been described. Data from non-small cell lung cancer, hepatocellular carcinoma, and cervical cancer (HeLa cell line) research suggest that metformin diminishes Nrf2 expression independently from the Keap1 protein (18). However, metformin also inhibits other signaling pathways, resulting in decreased Nrf2 activity (18). Moreover, metformin reduces the expression of the heme-oxygenase-1 protein, which sensitizes tumor cells to chemotherapy (18).

Based on histology, endometrial cancer has been classified into two broad pathogenic types: type 1 consists of grades 1 and 2 endometrioid endometrial carcinomas, whereas type 2 includes grade 3 endometrioid carcinoma and non-endometrioid endometrial carcinomas, such as serous and clear cell carcinomas (25). Typically, type 2 is more malignant and has a poorer prognosis (25). High Nrf2 expression has been demonstrated to have an association with type 2 endometrial cancer, whereas type 1 tissue samples do not exhibit such Nrf2 expression (13). Furthermore, high cytoplasmic Keap1 expression predicts poorer outcomes more accurately than the histological classification of endometrial cancer (14).

Our present study further confirmed the role of histology type as a key prognostic factor in OS, although we did not observe any association between Nrf2/Keap1 expression and survival. The modern approach to classifying endometrial carcinoma, which employs integrated genomic characterization, has identified four groups of carcinomas (26). Unfortunately, we were unable to reclassify our cohort according to this novel approach. However, our results are quite comparable to those of earlier studies that employed the same classification.

Reliable and extensive data on patient and tumor characteristics constitute one of the strengths of our study. Thorough documentation from our database yielded abundant information about age at diabetes and cancer diagnoses, type of diabetes, and ADM prescription, although ADM consumption was only assessed at the time of cancer diagnosis. Post-diagnostic adjustments to medication were not considered. Another strength of our study included our comprehensive tumor samples since, in nearly every case, we obtained the whole tumor as a pathological sample and endometrial biopsies were taken in only 11.6% of cases (*i.e.*, cases ineligible for surgery). All patients had access to the same resources and were treated according to national guidelines, which remained the same during the whole study period. The main shortcoming of our study was the small cohort size. The effect of this limitation was evident in the Cox regression model when the advanced stage exhibited no association with OS. This limitation could have been avoided had this study been conducted nationwide and not based on records from a single institution.

Conclusion

The role of MnSOD in endometrial cancer has not yet been widely studied. However, redox-regulating enzymes have previously been linked with chemoresistance and poorer prognosis in other cancer types. The present study suggests that in patients with T2DM and endometrial cancer, higher MnSOD expression in the tumors and serum is associated with better OS if the patient is consuming metformin.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Study conception and design: E.U., U.P., A.A. Acquisition of data: E.U. Revision of histological cancer data: A.A. Evaluation of immunohistoreactivity: A.A., E.U. Analysis and interpretation of data: E.K. Drafting of the manuscript: E.K. Revision of subsequent drafts: E.K., E.U., P.K., U.P., A.A. All Authors have read and agreed to the final version of the manuscript.

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