Scientific Electronic Archives
Issue ID: Sci. Elec. Arch. Vol. 18 (1)
January/February 2025
DOI: http://dx.doi.org/10.36560/18120252025
Article link: https://sea.ufr.edu.br/SEA/article/view/2025

ISSN 2316-9281

Anti-tumor effect of metformin in oncological patients: a systematic review

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Abstract. Metformin, a first-line drug used to treat patients with type 2 diabetes mellitus, has been studied for its metabolic, antiproliferative and pro-apoptotic effects in cancer patients. This study aimed to carry out a systematic review evaluating the effect of this drug in cancer patients, as well as to evaluate the clinical results obtained in cancer patients who used metformin independently or in association with other antineoplastic agents and to verify the possible mechanisms of action of metformin in tumor cells. Thus, a systematic review was carried out according to the protocol present in PRISMA, where the search string was defined using the PICOS approach. Articles in English and Portuguese, published from January 2002 to December 2022 in the following databases were selected: PubMed, Scielo, Lilacs, ScienceDirect, Springer and Wiley. 1,322 articles were identified through the search string and after excluding duplicate articles, it resulted in 1,311 articles for title and abstract reading. Next, 25 articles were included for complete reading and after the final analysis, 14 articles were included in the review. The selected articles indicate the potential effect of metformin in cancer patients, as well as better overall survival and disease-free survival. It was observed that metformin can exert its antiproliferative effect directly on tumor cells by activating the AMPK (AMP-activated protein kinase) enzyme which inhibits the mTOR (mammalian target of rapamycin) signaling pathway that is responsible for growth and cell proliferation. Furthermore, it was observed that metformin can act indirectly, which reduces circulating insulin levels and improves insulin sensitivity. Thus, it can be concluded that metformin has a significant antitumor effect in cancer patients, improving their overall survival, without triggering relevant adverse effects. The data demonstrate the potential antitumor effect of metformin both alone and in combination with other antineoplastic drugs.

Keywords: Metformin, Antitumor, Antineoplastic effect, Cancer, Systematic Review.

Introduction

Cancer is a public health problem and is one of the leading causes of death worldwide (BRAY et al., 2018). Cancer occurs due to a disorderly and malignant growth of abnormal cells, in which they proliferate in an exacerbated way, significantly increasing their quantity, thus forming a tumor that can spread to other regions of the body causing metastasis (INCA, 2020). In the year 2020, there were 19 million new cases of cancer in the world and 9.9 million patients died from this disease (GLOBOCAN, 2020a), and the estimate of cancer in the world for the years 2020 to 2030 is 24.6 million new cases (GLOBOCAN, 2020b).

Cancer is a multifactorial disease, where several intrinsic or extrinsic factors contribute to its development, among them obesity and type 2 diabetes mellitus (CHO et al., 2018; QUEIROZ et al., 2022).

When the diagnosis of cancer is confirmed, the treatment aims to promote the death of tumor cells or prevent their proliferation, thus reducing tumor growth. The treatment can be curative, control or palliative, generally also promoting the best quality of life for patients. There are three most common types of cancer treatment: chemotherapy, radiotherapy, and surgery. And in many cases these modalities are done together, for a better treatment of the patient (PORTH; MATFIN, 2011; QUEIROZ; ALEGRANCI; QUEIROZ, 2021).

Metformin is a drug widely used in the clinic, and it has become the first-line drug for the treatment of type 2 diabetes mellitus, as its toxicity effects are low and it differs from other antidiabetic drugs by normalizing blood glucose without causing hypoglycemia (ARAÚJO; BRITO; CRUZ, 2000). Still, it is indicated for the treatment of obesity, cardiovascular diseases and polycystic ovary syndrome (VIOLLET et al., 2012; KIRPICHNIKOV; MCFARLANE; SOWERS, 2002). Furthermore, studies are evaluating the effect of metformin in the treatment of patients with cancer (MITSUHASHI et al., 2014; SIVALINGAM et al., 2016).

Several studies carried out *in vitro* and *in vivo* demonstrate the possible antitumor effect of metformin on tumor cells. These studies demonstrate that metformin has a direct antineoplastic effect by activating the AMPK (adenosine-activated monophosphate kinase) protein, in which it inhibits the mTOR (mammalian Target of Rapamycin) pathway, which is responsible for cell growth and proliferation. Furthermore, metformin may also exert its antitumor effect by stopping the cell cycle and inducing apoptosis (QUEIROZ et al., 2014; QUEIROZ et al., 2015; CHEN et al., 2012; MOHAMMED, 2013). The indirect mechanism of metformin proposed by some authors indicates that the drug, by decreasing the hyperinsulinemia and insulin resistance, acting on glycemic control, leads to the reduction of mitogenic signaling pathways mediated by the insulin receptor. Thus, there is also a reduction in some signaling pathways activation, such as IGF-1 (insulin like growth factor 1), PI3K (phosphatidylinositol 3 kinase) and MAPK (mitogen-activated protein kinase) pathways, reducing cell growth and proliferation (BIRZNIECE et al., 2022; DELL'ATTI e GALOSI, 2018; DOWLING et al., 2015; KIM et al., 2015; QUEIROZ et al., 2014). It is observed that metformin is tolerable and can contribute to a better overall survival and improvement in the life expectancy of cancer patients, as well as acting in the reduction of the tumor. Thus, considering the potential antiproliferative effect of metformin, this systematic review aimed to carry out a review of studies in humans, evaluating the effect of this antidiabetic drug in cancer patients, evaluating the results of clinical studies carried out with cancer patients who underwent treatment with metformin, associated or not with other antineoplastic drugs.

In this context, the objective of the present systematic review was demonstrated in accordance with analysis of selected articles from the literature, the evidence relating to the antitumor effects of metformin in patients with cancer.

Several published surveys are available that cover different aspects of the association between metformin and cancer such as the literatures of Silva et al. (2021), Vieira et al. (2023) and Mallik and Chowdhury (2018). For example, Silva et al. (2021) demonstrated from the databases obtained in their integrative review, that metformin had a significant impact in patients with colorectal cancer. Where the antidiabetic drug can act synergistically with other chemotherapy drugs or also as a chemoprevention of colorectal cancer in these patients.

The study carried out by Viera et al. (2023) in Portugal, the collaborators showed in their systematic review that metformin was able to reduce the recurrence of prostate cancer by 20%, and that it was also effective in a better global and specific survival for prostate cancer in these patients. In the review by Mallik and Chowdhury (2018), it was possible to observe metformin in various types of cancer, such as lung, breast, pancreas, among others. Where, in the cited studies, the authors showed that this drug can act as monotherapy or in combination therapy by decreasing cell proliferation, as well as increasing the apoptosis of cancer cells (MALLIK; CHOWDHURY, 2018).

Although some studies have been demonstrating the effects of metformin in cancer patients, more studies should be developed, and reviews should be published to demonstrate this impact for the health. Thus, the outline of the contributions of this paper relative to the recent literature in the field can be summarized providing the influences of metformin on cancer, as well as the mechanisms associated.

To address these issues, general and specific questions related to the association between metformin and cancer were proposed: Does metformin present antitumor effects in oncological patients? Does metformin increase the overall survival or recurrence free survival of oncological patients? Has obesity, diabetes, and metformin

influence on oncological patients? What are the mechanisms associated with the antitumor effect of metformin in oncological patients? What are the side effects of metformin observed in oncological patients? These questions helped in the analysis and discussion of articles related to the research topic.

Material and Methods

A systematic review of the literature was carried out in order to analyze the antitumor effects of metformin in cancer patients. This review was conducted according to criteria present in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA Statement) (MOHER et al., 2009; PAGE et al., 2021). The process of defining the search string was carried out by searching scientific databases, correlating known terms such as synonyms, acronyms and combinations of words in the context of the work.

The approach used was PICOS, a strategy developed from the need for evidence-based practice (EBP), to support the definition of some subjects covered by PRISMA, such as objectives, research questions and eligibility criteria, and each letter refers to if to a component: participants (P), interventions (I), comparisons (C), outcomes (O) and chosen study design (S) (BERNARDO; NOBRE; JATENE, 2004) (SANTOS; PIMENTA; NOBRE, 2007).

Participants (P): individuals diagnosed with cancer and treated with metformin.

Intervention (I): treatment with metformin in cancer patients.

Comparison (C): comparing cancer patients treated with metformin with cancer patients who did not receive metformin treatment (control group); patients treated with metformin may or may not have concomitantly received treatment with other antineoplastic drugs.

Outcomes (O): analyze the antitumor effect, adverse effects, overall survival rate, recurrence free survival and clinical prognosis of patients treated or not with metformin.

Study design (S): the review includes non-randomized or randomized controlled clinical trials, cohort studies, case-control studies, cross-sectional and observational studies. All studies included in the work involve human beings.

Thus, for the development of this article, a systematic review was carried out with bibliographical research of the specific literature using the following search string in english: ("cancer" OR "neoplasm") AND ("metformin") AND ("antitumor" OR "antineoplastic/chemotherapeutic") AND ("human beings" OR "cancer patients") AND ("obesity") AND ("diabetes") and it's in Portuguese: ("câncer" OU "neoplasia") E ("metformina") E ("antitumoral" OU "antineoplásico") E ("sereshumanos" OU "pacientesoncológicos") E ("obesidade") E ("diabetes").

The selected articles were obtained from the following databases: MEDLINE/PubMed, Scielo, Lilacs, ScienceDirect, Springer and Wiley. Articles published in the period of 20 years, from January 1, 2002, to December 31, 2022, were considered. Mendeley Desktop was used as reference management software to organize the selected articles and carry out the selection process. All articles were independently reviewed by five reviewers, who verified their relevance to the scope of this review.

Inclusion criteria were clinical trial articles (randomized or non-randomized), cohort study, case-control study, cross-sectional study and observational study; articles made with human beings; articles that contain in their sample patients diagnosed with cancer and treated with metformin (alone or in combination with other antineoplastics drugs); articles published in English and in Portuguese; and articles published from 2002 to 2022. Exclusion criteria were cancer patients treated with metformin and treated with other antidiabetic/antihyperglycemic drugs; patients with cancer and presenting concomitantly an autoimmune disease or using immunosuppressive drugs, except for glucocorticoids; and patients under 18 years of age.

In the first step, all duplicated articles in the database were removed and the articles found were selected by reading the title and abstract in accordance with the inclusion and exclusion criteria. In the second stage, the complete reading of the selected articles and the evaluation of their methodological quality were carried out. The analysis and selection of articles was carried out from January 1st, 2023 to March 30rd, 2023.

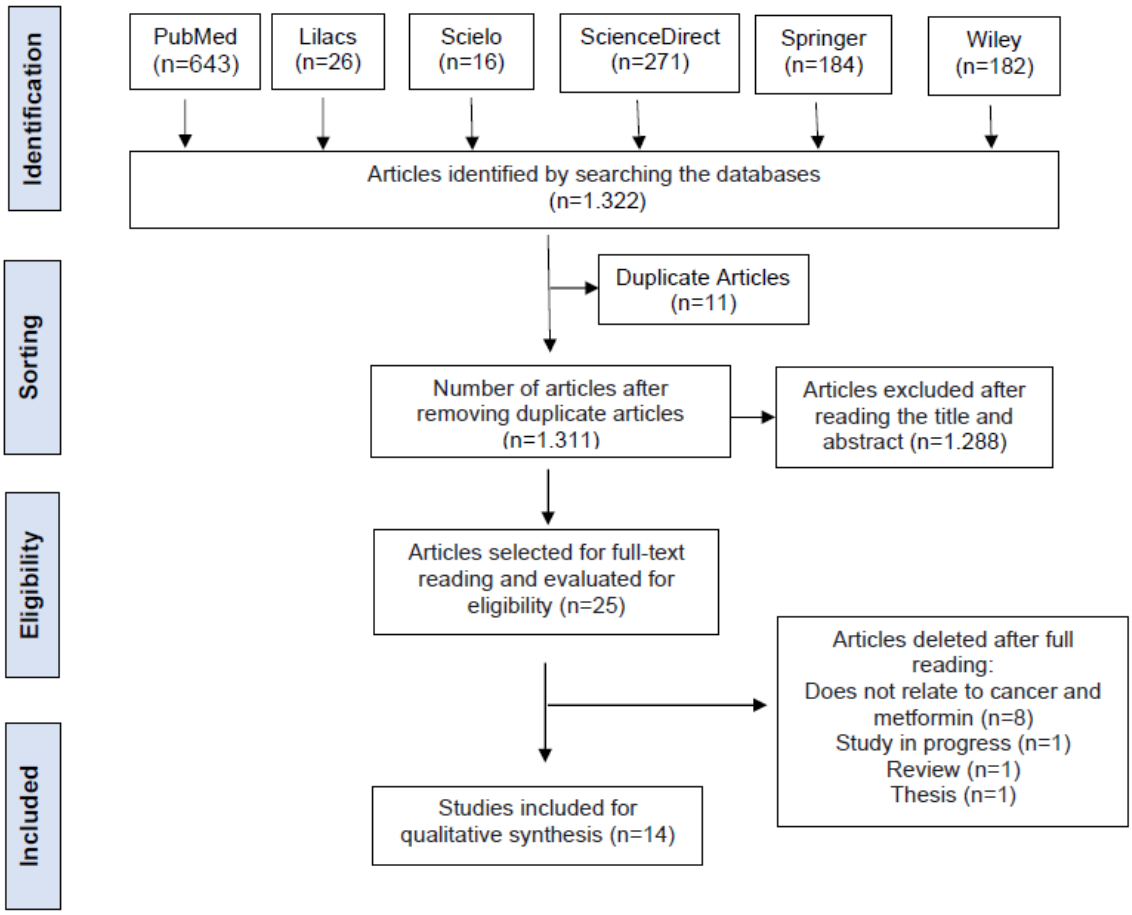

Figure 1. PRISMA flow diagram with the steps in the article selection process.

Results and discussion

Initially, 1,322 articles were identified through the search string, in the selected database: PubMed (643), Lilacs (26), Scielo (16), ScienceDirect (271), Springer (184) and Wiley (182). The first selection step was the exclusion of duplicate articles, resulting in 1,311 articles for title and abstract reading, in which 25 articles were included for full reading based on the inclusion and exclusion criteria. After reading the 25 selected articles, 14 articles were included for the qualitative analysis of this work(Figure 1). General information regarding the selected articles is present in Table 1.

Among the 14 selected articles, 11 articles demonstrated that metformin has a significant antitumor effect in cancer patients undergoing active treatment (Table 1). Among the types of cancer evaluated, the most studied and cited were breast cancer (6), prostate cancer (3), endometrial cancer (3) and lung cancer (2) (Table 2). Also, among the 14 selected articles, 8 articles demonstrated that metformin increased the recurrence free survival (RFS) and the overall survival of cancer patients (Table 1).

It was observed that the main types of studies performed were retrospective cohort studies (9 – 64.3%), cross-sectional (2 – 14.29%), randomized clinical trial (2 – 14.29%), and related case (1 – 7.44%) (Table 2).

The works were carried out in the following countries: United States (4), Korea (2), Japan (1), Australia (1), Canada (1), Norway (1), Iran (1), Egypt (1), Italy (1) and Spain (1). It was observed that no study carried out in Brazil was found in the database, as well as no country in South America (Table 1).

It was noted that a total of 289,394 cancer patients were included for the studies. Of these, 152,089 patients were diabetic (52.55%), and 130,095 were using metformin (85.54%), the other diabetic patients were using other antidiabetics drugs.

According to the 14 selected articles, only 6 articles reported the dose of metformin used in cancer patients. It was observed that the drug dose ranged from 250 mg per day to a maximum of 2,000 mg per day (BARAKAT et al., 2020; BIRZNIECE et al., 2022; CHO et al., 2018; DOWLING et al., 2015; KO et al., 2015; SATO et al., 2017).

Does metformin present antitumor effects in oncological patients?

Most of the studies agree on the action of the antitumor effect of metformin in cancer patients, and of the 14 articles selected, 11 articles demonstrated that metformin has a significant antitumor effect in cancer patients undergoing active treatment (Table 1). Furthermore, studies have shown that metformin

may have a direct and indirect antineoplastic effect, providing a protective effect, a better prognosis and an improvement in patient survival (BERGAMINO e al., 2019; CHO et al., 2018; KO et al., 2015).

In the population-based retrospective cohort study by Cho et al. (2018), the authors verified the incidence of prostate cancer in a population diagnosed with type 2 diabetes mellitus, where they reported from reports that the average dose of metformin in patients was 531 mg/day, in which metformin contributed to maintaining the individual's body weight without promoting weight loss or gain. Also, in that study, there was a 31% reduction in the incidence of prostate cancer in patients using metformin.

In Egypt, a randomized clinical study conducted by Barakat and collaborators (2020), showed that neoadjuvant metformin to chemotherapy did not demonstrate an improvement in clinical and tumor responses, however, the results were increased regarding the evaluation of the complete pathological response in the metformin group compared to the control group.

A retrospective study of 351 women with endometrial cancer and obesity, of which 64 were using metformin, reported that patients not using metformin had a 15% recurrence rate for all types of endometrial cancers, compared with patients using the drug, 4.7% (p=0.027). Still, the authors suggest that metformin can be an ally in adjuvant therapy in endometrial cancer in women with obesity, as well as an endometrium cancer preventive agent (HALL et al., 2016).

In a case report, a patient with endometrial cancer and type 2 diabetes mellitus who used metformin for 45 months maintained a good quality of life, with no signs of tumor progression, demonstrating that metformin can be beneficial in cancer prevention, as also in the treatment (SATO et al., 2017). It is noteworthy that in this study the patient was treated only with metformin since she could not undergo chemotherapy or radiotherapy due to her clinical condition after hysterectomy, where she had chronic inflammation in the peritoneum and perforation in the gastrointestinal tract.

Does metformin increase the overall survival or recurrence free survival of oncological patients?

As previously mentioned, it was observed that of the 14 selected articles, 8 articles demonstrated that metformin increased disease-free survival and overall survival of cancer patients (BEHROUZI; MOHAGHEGHI; SADIGHI, 2017; BERGAMINO et al., 2019; DOWLING et al., 2015; HALL et al., 2015; KIM et al., 2015; KO et al., 2014) (Table 1).

Ko et al (2014) reported that patients who used metformin had a significant improvement in overall survival (p=0.003) and disease-free survival (p=0.01). Similarly, a retrospective study conducted in Korea reported that patients in the metformin group had increased cancer-specific survival and disease-free survival (p=0.058) compared to the group without metformin (KIM et al., 2015). Bergamino et al. (2019) observed in their retrospective study that patients who used metformin had a higher overall survival compared to patients who used insulin (p=0.001).

Another study evaluating overall survival, conducted by Brancher et al. (2022), in Norway, evaluated pre-diagnosis use of metformin (use of the drug at least one year before cancer diagnosis) in which there was no significant difference in overall survival between users and non-users of metformin. However, the authors reported that the use of the drug post-lung cancer diagnosis had a better overall survival for all patients.

Table 1. Description of selected articles

Authors: KO, Emily M. et al. (2014)	**Type of study:** Retrospective cohort
Country (n): USA (n = 1495)	**Type of cancer:** Endometrial cancer
Antitumor effect and mechanism of action: Metformin acts by directly activating AMPK and inhibiting the mTOR pathway. And indirectly, by decreasing hyperinsulinemia, hepatic gluconeogenesis and insulin sensitivity.	
Clinical prognosis and survival: There was an improvement in OS and RFS. Metformin appears to improve survival and death from all causes	
Authors: BIRZNIECE, Vita et al. (2022)	**Type of study:** Crossed and randomized
Country (n): Australia (n = 15)	**Type of cancer:** Prostate cancer
Antitumor effect and mechanism of action: ADT therapy increases many factors, such as hyperinsulinemia, responsible for increasing circulating IGF. The drug metformin decreases circulating IGF-2 and IGFBPs, as well as STC2.	
Clinical prognosis and survival: 11 of the 15 patients had HOMA IR>1.9 indicating insulin resistance. After metformin treatment, HOMA IR and liver resistance fell (p=0.05).	
Authors: CHO, Yoon Young et al. (2018)	**Type of study:** Retrospective cohort
Country (n): Korea (n = 256.906)	**Type of cancer:** Prostate cancer
Antitumor effect and mechanism of action: Circulating insulin stimulates the mTOR pathway and metformin has indirect effects on this pathway by decreasing hepatic hyperinsulinemia and gluconeogenesis. As well as directly activating AMPK and consequent inhibition of mTOR.	
Clinical prognosis and survival: There was a 31% reduction in prostate cancer in patients using metformin (p=0.01). The use of the drug for a longer period (1084-2094).	

Authors: KO, Kwang-Pil et al. (2015) **Type of study:** Randomized double-blind, placebo-controlled
Country (n): USA (n = 105) **Type of cancer:** Breast cancer
Antitumor effect and mechanism of action: Metformin, by decreasing hyperinsulinemia, decreases circulating IGF and IR (which are responsible for cell proliferation. It also inhibits the mTOR pathway by activating AMPK.
Clinical prognosis and survival: Patients who used metformin 1,000mg/day had a reduction of 14, 3% circulating insulin Glucose and HbA1c levels decreased

Authors: SATO, Emi et al. (2017) **Type of study:** Case Report
Country (n): Japan (n = 1) **Type of cancer:** Endometrial cancer
Antitumor effect and mechanism of action: Metformin may have antitumor effects by arresting the cell cycle. This drug decreases mRNA expression in reverse transcriptase in human tolomerase. And also, the direct effects of activating the AMPK pathway, which inhibits mTOR, decreasing cell growth and proliferation.
Clinical prognosis and survival: Patients remained disease-free for a long period. He had no complaints and maintained good quality of life without tumor progression

Authors: JIRALERSPONG, S. et al. (2013) **Type of study:** Retrospective cohort
Country (n): USA (n = 6.342) **Type of cancer:** Breast cancer
Clinical prognosis and survival: There was no significant difference in RFS between diabetic and non-diabetic patients who were on treatment. In this case, metformin may have attenuated differences in patient survival

Authors: BEHROUZI, B.;MOHAGHEGHI, M. A.;SADIGHI, S. (2017) **Type of study:** Retrospective cohort
Country (n): Iran (n = 1.021) **Type of cancer:** Breast Cancer
Antitumor effect and mechanism of action: Metformin can inhibit the Ki-67 marker that is present in breast cancer, therefore decreasing cell proliferation. This drug may also have its effect by decreasing hyperinsulinemia and consequent decrease in cell proliferation.
Clinical prognosis and survival: There was a greater impact in diabetic patients with high BMI (which can be improved with the use of metformin).

Authors: HALL et al. (2015) **Type of study:** Retrospective cohort
Country (n): USA (n = 351) **Type of cancer:** Endometrial cancer
Clinical prognosis and survival: Patients that used metformin had a lower recurrence of cancer when compared with patients without treated with metformin(4.7% vs. 15%, respectively).

Authors: BRANCHER et al. (2021) **Type of study:** Retrospective cohort

Country (n): Norway (n = 22.324) **Type of cancer:** Lung Cancer
Antitumor effect and mechanism of action: Metformin can have its direct effects, by activating the AMPK pathway, which inhibits the mTOR pathway and consequent decrease in cell proliferation, or indirectly by decreasing hyperinsulidemia, improving glycemic control, as well as decreasing the inflammatory response
Clinical prognosis and survival: All patients who used metformin after diagnosis of lung cancer had a better survival (OS). Prediagnosis use was meant for LCSS (Lung Cancer Specific Survival).

Authors: GALOSI, Andrea B. (2018) **Type of study:** Retrospective cohort
Country (n): Italy (n = 551) **Type of cancer:** Prostate cancer
Antitumor effect and mechanism of action: Metformin inhibits other signaling pathways by reducing hyperinsulinemia, such as IGF and PI3K-AKT/IR. Another suggestion would be the inhibition of the mTOR pathway, by directly activating AMPK. By inhibiting these signaling pathways, this drug exerts its antiproliferative effect
Clinical prognosis and survival: Metformin duration <2 years influenced prostate cancer incidence (78%) as well as Gleason score >7 (89%) compared with duration greater than 2 years

Authors: KIM, Hee Jeong et al. (2015) **Type of study:** Retrospective cohort
Country (n): Korea (n = 6.967) **Type of cancer:** Breast Cancer
Antitumor effect and mechanism of action: Metformin can act directly by activating AMPK and this inhibits the mTOR pathway. As well as suppressing PI3K/AKT/mTOR signaling pathways by indirectly inhibiting insulin and IGF receptors
Clinical prognosis and survival: There was an increase in CSS and DFS (p=0.058) in the metformin group. As well as improvement in DFS in subgroups with positive HER-2 and positive hormone receptor. Metformin 1000mg groups decreased glucose and HbA1c (p= 0.094 and 0.073) compared to metformin 500mg and placebo

Authors: BARAKAT, Hadeer Ehab et al. (2022) **Type of study:** Randomized clinical Trial
Country (n): Egypt (n = 74) **Type of cancer:** Breast Cancer
Antitumor effect and mechanism of action: Metformin may suppress (PI3K)/AKT/mTOR signaling pathways or IGF-1 signaling by decreasing hyperinsulinemia, especially in HER2+ patients
Clinical prognosis and survival: Neoadjuvant therapy with metformin has not been shown to improve clinical and tumor responses. However, the results were increased in the metformin group compared to the control group

Authors: DOWLING, Ryan JO et al. (2015) **Type of study:** Neoadjuvant clinician
Country (n): Canada (n = 39) **Type of cancer:** Breast Cancer
Antitumor effect and mechanism of action: Metformin indirectly decreases insulin receptor expression and PI3K/PKB/Akt/mTOR and Ras-MAPK signaling pathways, which are present in mitogenic pathways. The presence of OCT1 in all tumors indicates greater activation of AMPK directly activated by metformin
Clinical prognosis and survival: A 10% decrease in circulating insulin was observed in patients who used metformin, and patients who had a greater reduction in PKB/AKT phosphorylation (p=0.02) had a greater reduction in cell proliferation

Authors: BERGAMINO, Milana et al. (2019) **Type of study:** Retrospective cohort
Country (n): Spain (n = 170) **Type of cancer:** Lung Cancer
Antitumor effect and mechanism of action: BERGAMINO et al. agree with other authors that metformin exerts its antitumor effect directly (by activating AMPK and this inhibiting the mTOR pathway), and indirectly by decreasing hyperinsulinemia
Clinical prognosis and survival: It was observed that patients using metformin (n=20) had a longer overall survival compared to patients using insulin (p=0.001).

Has obesity, diabetes, and metformin influence on oncological patients?

Reading the articles, it was observed that type 2 diabetes mellitus is a comorbidity that is accompanied by almost all types of cancer and that, when left untreated, is related to a worse prognosis and a shorter survival for patients (KO et al., 2014; BIRZNIECE et al., 2022; JIRALERSPONG, S. et al., 2013).

JIRALERSPONG et al. (2013) reported that patients with obesity were more likely to die and have worse disease-free survival than eutrophic patients (p=0.022), and patients with breast cancer who were diabetic were more likely to die and had a shorter survival compared to non-diabetic patients (p= 0.007).

Ko et al. (2014) reported that in a cohort of 1495 women with endometrial cancer, 363 were diagnosed with type 2 diabetes mellitus and that only 55% of these patients' used metformin and had better overall survival, the rest used other types of antidiabetics. Similarly, in a cross-sectional study by Birzniece et al. (2022), patients using metformin had less weight gain and a decrease in hepatic insulin resistance compared to the placebo group (p<0.05), contributing to an antiproliferative effect of the drug.

Table 2. Number of articles selected according to the variables: year of publication, type of study, types of cancer, adverse effects.

Variable	Number (percentage)
Year of publication	
2002 – 2005	0 (0,00%)
2006 – 2009	0 (0,00%)
2010 – 2013	2 (15,38%)
2014 – 2017	6 (46,15%)
2018 – 2022	6 (46,15%)
Type of study	
Retrospective cohort	9 (64,29%)
Prospective Cohort	0 (0,00%)
Case control	0 (0,00%)
Transversal	2 (15,38%)
Population base	0 (0,00%)
Clinical study - Randomized	2 (15,38%)
Clinical study - Non-randomized	0 (0,00%)
Others	1 (7,69%)
Types of cancer	
Breast Cancer	6 (46,15%)
Prostate cancer	3 (23,08%)
Endometrial cancer	2 (15,38%)
Lung Cancer	2 (15,38%)
Adverse effects	
Gastrointestinal	
Others	

The randomized placebo-controlled study by Ko et al. (2015), where 105 women with breast cancer with obesity and who were not diabetic, were included and randomly divided into three groups: placebo, metformin 500mg and metformin 1000 mg, with time 6-month treatment period, where the metformin 1000 mg group achieved a significant decrease in glucose and HbA1c (glycated hemoglobin) (p=0.008 and p=0.009, respectively) compared to the other groups. Also, metformin at a dose of 1000 mg was effective in reducing circulating insulin levels by 14.1%, which is an important factor because hyperinsulinemia can leads to cell proliferation, and if metformin acts in this way by reducing insulin levels, can decrease cell proliferation indirectly.

Another retrospective study carried out in Iran in a cohort of 1,021 women with breast cancer, 218 had type 2 diabetes mellitus. Also, patients with estrogen receptor (ER)/progesterone receptor (PR) positive, HER-2 (Human epidermal growth factor receptor type 2) negative and diabetes 1.7 and 1.8 times more likely to have relapse or mortality (BEHROUZI, MOHAGHEGHI, MOHAMMAD, SADIGHI, 2017). In agreement with this finding, metformin had a significant effect on the survival of patients with ER/PR-positive and HER-2-positive breast cancer. Type 2 diabetes mellitus patients receiving metformin and neoadjuvant chemotherapy have a higher pathological response rate compared to diabetic patients not taking metformin (KIM et al., 2015). Similar to this one, in a neoadjuvant clinical study, HER-2 positive patients had a better pathological complete response (PCR) (p=0.044) compared to the other subtypes (BARAKAT et al., 2020).

What are the mechanisms associated with the antitumor effect of metformin in oncological patients?

Most studies agree on the action of the antitumor effect of metformin treatment in cancer patients, as it comes from the ability of this drug to generate an improvement in metabolism parameters, glycemic index and insulin resistance in patients, which in fact would by itself generate an antiproliferative action. Metformin may have a direct and indirect antineoplastic effect, providing a protective effect, a better prognosis, and an improvement in patient survival (BERGAMINO et al., 2019; BRANCHER et al., 2021; CHO et al., 2018; KIM et al., 2015; KO et al., 2014; KO et al., 2015; SATO et al., 2017).

Birzniece et al. (2022) performed a randomized study with patients with prostate cancer for 6 weeks, in which the maximum dose of the metformindrugwas 1500 mg and analyzed the possible effect of metformin on the IGF-IGFBPs (IGF Binding Proteins) pathway in patients with prostate cancer. In this study, the main cancer therapy, ADT (androgen deprivation therapy), is linked to the development of obesity, insulin resistance, and hyperinsulinemia. Hyperinsulinemia can stimulate carcinogenesis directly or indirectly through the insulin receptor or through IGFs. Insulin acts reducing the IGFPB levels and thus increasing the bioavailability of IGFs, increasing the quantity of circulating free IGF contributing with its effects stimulating cell proliferation and inhibiting apoptosis (SANDHU; DUNGER; GIOVANNUCCI, 2002).

In this study, Birzniece et al. (2022) demonstrated that metformin decreased significantly the IGF-2 and IGFBP-3 levels, and HOMA IR index, indicating an antiproliferative effect of the drug, as well as a significant improvement in hepatic insulin resistance. In addition, Birzniece et al. (2022) demonstrated a reduction in circulating STC2 (Stanniocalcin 2) levels ($p<0.05$) of patients with prostate cancer treated with metformin. It is known that overexpression of STC2 is related to cancer progression. STC2 was related to the HOMA IR index, indicating that the better insulin sensitivity, the greater the reduction of STC2. Therefore, as STC2 can increase cancer growth, this would result in a possible mechanism of metformin's antitumor effect.

In a clinical study conducted in Canada, 39 women diagnosed with breast cancer were given metformin 500mg three times a day for 2 weeks, where metformin significantly decreased insulin receptor (IR) signaling in tumor cells (p=0.04), as well as phosphorylation of PKB/AKT (protein kinase B/) and ERK1/2 (mitogen-activated kinases MPKs). All tumors expressed OCT1 (organic cation transporter 1), indicating an uptake of metformin by tumor cells. Short-term administration of metformin induces tumor-specific changes in insulin receptor (IR) expression and cell signaling, which are consistent with the drug's beneficial anticancer effects (DOWLING et al., 2015).

Thus, the indirect mechanism of metformin proposed by some authors indicates that the drug, by decreasing hyperinsulinemia and hepatic insulin resistance, acting on glycemic control, consequently, leads to the reduction of mitogenic signaling pathways mediated by the insulin receptor, as IGF-1, AKT/PI3K (Phosphoinositide 3-kinase) and MAPK (Mitogen-activated protein kinase) pathways (DOWLING et al., 2015; KIM et al., 2015; DELL'ATTI and GALOSI, 2018).

What are the side effects of metformin observed in oncological patients?

Among the possible adverse effects that metformin can cause, in the study by Barakat et al. (2020) there were gastrointestinal effects, especially nausea, vomiting and diarrhea. However, metformin improved some side effects of neoadjuvant chemotherapy, such as neuropathy, bone pain, arthritis and myalgia. In the study by Birzniece et al. (2022), only one patient had gastrointestinal adverse effects, and the dose of metformin was reduced in that patient. In the study by Kim et al. (2015), only one patient in the metformin 500mg group had dizziness.

Discussion

It was observed that there are few clinical studies regarding metformin in the treatment of cancer, since, of the 14 articles selected for analysis, 9 (64.29%) were retrospective studies, and only 4 evaluated metformin in practice, in which only 3 articles compared the effects of the drug with the placebo group. This is important to assess whether metformin actually has an antitumor effect. Still, in relation to the years of publication, it was observed that only in the last 10 years did they begin to evaluate the antineoplastic effect of metformin in humans, obtaining a greater growth of studies from the year 2014 (50,0%).

This shows the scarcity of clinical studies evaluating the effect of metformin in cancer patients and demonstrates the importance of developing new studies, especially clinical studies, carried out in this area.

Metformin is a first-line drug to treat type 2 diabetes mellitus, and is being widely studied in vitro and in vivo, with the aim of evaluating whether this drug really has antitumor effects KIRPICHNIKOV; MCFARLANE; SOWERS, 2002; QUEIROZ et al., 2014; QUEIROZ et al., 2015; SAHRA et al., 2008; VIOLLET et al., 2012).

A study conducted by Chen et al. (2012) analyzed whether metformin at a concentration of 10 mM (millimolar) inhibits the growth of cells (HTh74 and HTh74dox) of thyroid cancer. In that study, metformin induced apoptosis and blocked the cell cycle in the G0-G1 phase. Also, metformin activated AMPK in these cells, and as a result, the mTOR signaling pathway was reduced.

Sahra et al (2008) carried out a study in thyroid cancer cells, and also showed the arrest of the cell cycle in the G0-G1 phase mediated by metformin, however, the drug did not induce apoptosis. Similarly, Queiroz et al. (2014) demonstrated that metformin at the same concentration promotes cell cycle arrest in the G0-G1 phase of MCF-7 breast cancer cells, as well as apoptosis and cell necrosis. There was an inhibition of p70S6K protein (ribosomal protein kinase S6), which may be an explanation for metformin-mediated apoptosis. Furthermore, Queiroz et al (2014) reported an increase in the AMPK pathway activation, and mTOR inhibition. Another mechanism suggested by the authors is the expression of the insulin receptor, which activates some pathways responsible for cell growth and proliferation, such as the MEK/ERK1/2 (extracellular signal-activated protein kinase 1 and 2)/MAPKpathways. mitogen-activated), and in this study there was a significant reduction in IR and ERK1/2, which suggests an antiproliferative effect of metformin (Figure 2).

An in vitro study conducted by Teixeira et al. (2013) evaluated whether neoadjuvant metformin improves cell growth and proliferation in the NCI-H460 non-small cell lung cancer cell line (NSCLC). The authors performed a combination of metformin (60.58 Mm) and cisplatin (0.19 mm), in which better antiproliferative effects were obtained than the half dosage. Another combination used was metformin (30.39mm) and etoposide (0.18mm), which was also better at reducing cell proliferation than at reduced doses. This study demonstrated that metformin in combination with other chemotherapeutic agents has an antineoplastic effect, being able to increase cellular death and act synergistically.

Queiroz et al (2015) conducted a study in obese (monosodium glutamate-induced) and non-obese rats, where the Walker-256 tumor (breast cancer strain) was inoculated, and on the same day treatment with metformin started of 300 mg/kg for 15 days. The authors demonstrated that tumor development was greater in obese rats than in the control group, and in the obese group with metformin there was tumor reduction. Furthermore, treatment with metformin decreased the IR/MERK/ERK 1/2 pathway in the control and obese groups compared with the groups without metformin, which may have contributed to the reduction in tumor growth.

FOXO (Forkhead transcription factor) proteins (FOXO1, FOXO3a, FOXO4, FOXO6) are related to tumor suppression and longevity, where they regulate genes associated with stress, metabolism, cell cycle arrest and apoptosis (CALLE and KAAKS, 2004; CHIACCHIERA and CRISTIANO, 2009; GREER, BANKO and BRUNET, 2009). FOXO3a can induce transcription of genes that are involved in cell cycle arrest and death, shrinking the tumor (CALLE and KAAKS, 2004; CHIACCHIERA et al., 2009).

Thus, some authors agree on the direct mechanism of metformin in tumor cells, where it activates AMPK and consequently inhibits the mTOR signaling pathway (Figure 2) (CHEN et al., 2012; QUEIROZ et al., 2014; QUEIROZ et al., 2015).

In this systematic review, it was possible to observe that studies carried out in humans also demonstrated an important antitumor effect of metformin and that this was associated with AMPK activation and mTOR inhibition, as well as related to the reduction of circulating insulin levels and improvement of insulin sensitivity in cancer patients (Figure 2).

A study conducted by Sivalingam and collaborators (2016) verified the antitumor effect of metformin in endometrial cancer, in order to verify a reduction in the Ki-67 proliferation marker after hysterectomy. A cohort of 12 women without metformin and 28 women receiving 850 mg of metformin twice daily from 7 to 30 days until the night before surgery. The authors reported that there was a 17.2% decrease in Ki-67 after the use of metformin, compared to patients who did not use it, where it remained static and increased by 28% (7 of 12 patients). In this study, a reduction in the mTOR signaling pathway was observed in patients who used metformin.

The phosphorylated 4E binding protein (P-4EBP1) is expressed in several types of tumors, and

when phosphorylated it can contribute to cell growth and proliferation (ZANCO, EFEYAN, SABATINI, 2011; ZHANG et al., 2018). Curiously Sivalingam et al. al (2016) reported a significant decrease in P-4EBP1 (p=0.045).

Similarly to the study by Sivalingam (2016), a study conducted in Japan recruited 40 women with endometrial cancer who had a scheduled hysterectomy, in which 35 agreed to participate in the study and 4 patients refused due to side effects (nausea). In that study, the initial dose was 750 mg per day, reaching 2,250 mg per day for 4 to 9 weeks (MITSUHASHI et al., 2014).

Mitsuhashi et al. (2014) reported that 28 patients had a 42.2% reduction in Ki-67 and reduced topoisomerase II expression by 36.4% in 25 patients. This study is similar to the in vitro study by Queiroz (2014) where metformin increased circulating levels of the AMPK pathway and mTOR pathway inhibition, as well as decreased MAPK expression. And it is related to the study carried out in mice by Queiroz (2015), the authors observed an activation of p27 after a decrease in ERK 1/2 by 65.8%, which results in the arrest of the cell cycle. Still, there was also inhibition of cyclin D1 in patients who used the drug (MITSUHASHI et al., 2014).

In a clinical trial, patients with breast cancer who received metformin adjuvant to chemotherapy had a better radiological response rate compared to the control group (p=0.002). Furthermore, the metformin group had better progression-free survival compared to the no-metformin group (4.4 vs 5.1 months, respectively) (RABEA; HASSAN; ELBERRY, 2021).

Thus, it is observed that the studies carried out in cell lines, in animals and in humans are similar. The authors agree on the mechanism of action in which metformin can exert its antitumor effect, in which the main direct mechanism would be the activation of AMPK and, consequently, a decrease in the activation of the mTOR pathway. Or indirectly, by reducing hepatic gluconeogenesis and circulating insulin levels (Figure 2 and 3).

This work had some limitations: 1) we only evaluated articles published in the PubMed, Scielo, Lilacs, Wiley, Springer and ScienceDirect databases, therefore, other articles performed in humans that were in other databases may have been disregarded. 2) We evaluated only articles in English and Portuguese, so articles in other languages that could be relevant may have been excluded.

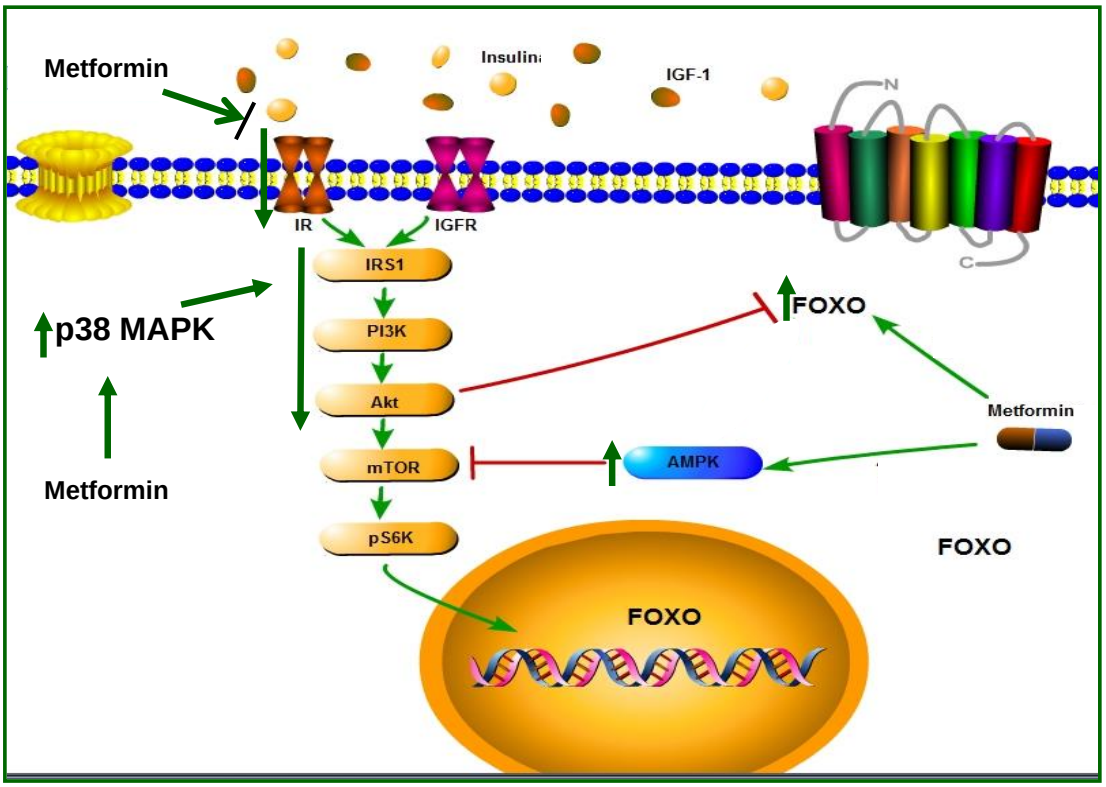

Figure 2. Schematic representation of the mechanism of action of metformin in cancer cells.

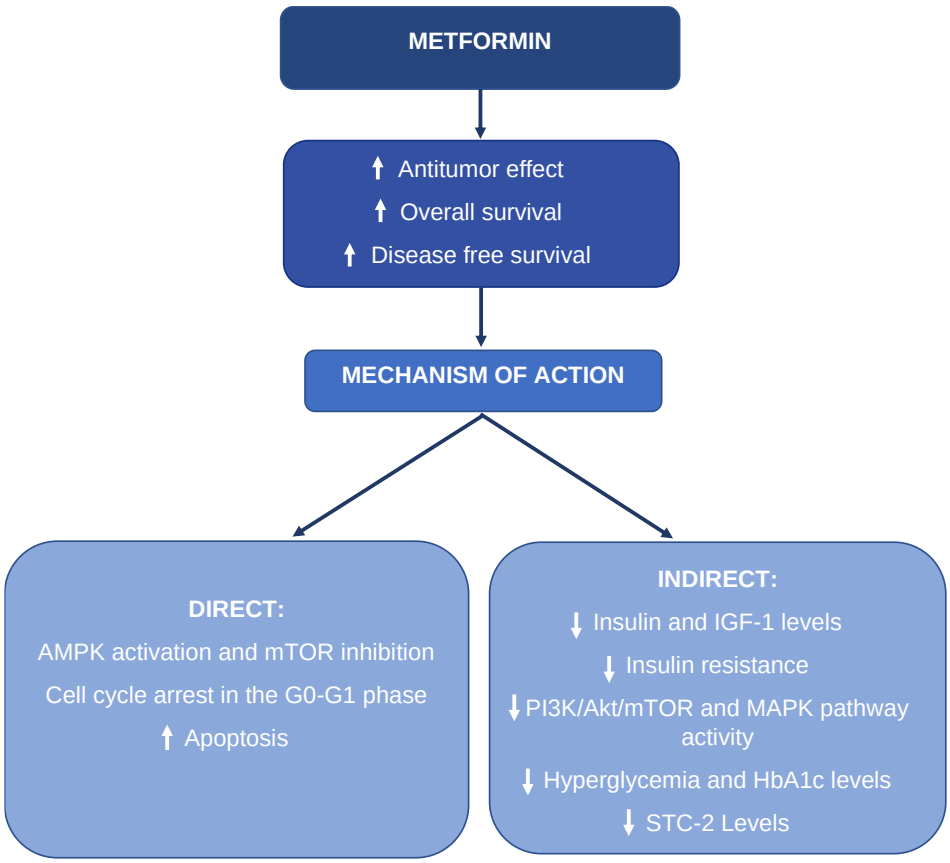

Figure 3. Schematic representation of the association between metformin and cancer. Antitumor effect of metformin in oncological patients and its mechanisms of action.

Conclusion

As observed throughout the work, it can be concluded that metformin has a potential antitumor effect, reducing tumor development, improving life expectancy, disease-free survival and overall survival of cancer patients, without triggering relevant adverse effects. Furthermore, it was observed that metformin has an antiproliferative effect and a lower incidence of cancer in patients who use the drug, and these effects are related to the action of metformin activating AMPK, reducing circulating insulin levels and improving insulin resistance (Figure 3). However, it was observed that the works carried out in humans are scarce, being necessary more clinical studies carried out in cancer patients to better evaluate the antitumor effect of metformin in these patients.

Competing Interests

The authors declare no conflicts of interest.

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