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SCDU di Endocrinologia
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CLINICAL STUDY, OBSERVATIONAL, NON-PROFIT

TITLE: Type 2 Diabetes and Cancer: Phenotyping of patients followed in a tertiary diabetes center

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SUMMARY

1. INTRODUCTION

The relationship between type 2 diabetes (T2D) and cancer has gained considerable attention and importance in recent years. These are two highly prevalent conditions in the global population with a significant impact on the healthcare system: in fact, estimates from the International Diabetes Federation (IDF) show that approximately 537 million people suffer from diabetes, the majority of whom have T2D. (1)

The association between the two conditions has been widely studied, revealing how some metabolic processes can be common and influence each other. Hyperglycemia itself, caused by poorly controlled diabetes, can result in the development of a chronic low-grade systemic inflammatory state; this environment arises as a consequence of chronic glucose stimulation of certain mechanisms, including the calcium signal, which leads to the release and increase of inflammatory cytokines and consequent inflammatory cells. (2) Furthermore, in addition to perpetuating the inflammatory state, there is an escalation in ROS (reactive oxygen species) production, triggering oxidative stress and DNA damage, thus advancing cancer development. (3)

Concerning the chronic inflammatory state, it is also crucial to consider the role of obesity, being one of the most important and studied common elements between the two pathologies. In response to hypoxia and oxidative stress, adipose tissue produces high levels of leptin, a pro-inflammatory adipokine. (3, 4) This overproduction can disrupt the equilibrium between leptin and other adipokines, encouraging a state of meta-inflammation that enhances cancer development. It promotes processes such as epithelial-mesenchymal transition (EMT) and cellular adhesion to the extracellular matrix (ECM) (3), while also facilitating cancer immune evasion. (5) It is therefore important to understand how the increase in leptin related to obesity, a risk factor often present in the diabetic population, also plays a significant role in cancer.

Finally, another factor contributing to this inflammatory microenvironment and cytokine production is dyslipidemia, particularly the increase in fatty acids. (3)

Not surprisingly, in addition to these molecules, the microbiota also plays a significant role. Dysbiosis, implicated in various diseases, has been associated with cancer as well, owing to its capacity to influence the genomic stability of host cells and modulate numerous signaling pathways. Consequently, a correlation has been demonstrated between reduced richness of intestinal microbiota and metabolic dysfunctions, including insulin resistance and inflammation. (6)

Another important characteristic of T2D, namely hyperinsulinemia, can also be part of and contribute to the development of cancer. High levels of insulin stimulate not only the insulin receptor but also insulin-like growth factor (IGF-1) (4), which is implicated in the onset and development of various types of cancers. Studies on murine models have shown how the administration of IGF-1 not only promotes the proliferation of cancer cells but also their ability to metastasize. (7)

In addition to this hormone, mTOR is also a central molecule, which regulates processes such as growth and metabolism, (8) thus potentially playing a role in both pathologies. Dysregulations of metabolism with increased nutrient intake, as in the majority of patients with T2D, lead to hyperactivation and stimulation of this signal, which contributes to the worsening of diabetic pathology with increased insulin secretion. (9)

The same signal can activate various mechanisms, including the MAP kinase pathway, which is frequently subject to mutations in some types of cancers. Furthermore, hyperactivation of the mTOR pathway contributes to the promotion of pro-oncogenic proteins, which control fundamental processes for the survival of cancer cells, such as angiogenesis, metabolism, and the capacity for metastatic spread. (10)

An interesting metabolic phenomenon is the Warburg effect, which explains how, despite ample oxygen availability, cancer cells prefer to generate energy through glycolysis, which requires increased glucose uptake, making the hyperglycemic environment advantageous for cancer cells. Moreover, this process leads to increased production of a precursor of advanced glycation end products (AGEs), which means that the Warburg effect not only affects the metabolism of cancer cells but also contributes to the development of diseases by promoting the accumulation of AGEs. (3)

Finally, even diabetes drugs could be implicated. One extensively studied drug is metformin, which has shown potential as an antineoplastic agent in diabetic subjects, unlike exogenous insulin, which seems to act as a growth factor potentially implicated in cancer proliferation. (11)

All these common metabolic processes, as well as others, highlight the importance of carefully characterizing the population of diabetic patients also affected by cancer in order to understand the associated risk factors. As cancer represents a complication of diabetes, this study underscores the primary objective, which is to emphasize the necessity for tailored prevention strategies in clinical settings. These strategies aim to address specific diabetes-related risk factors that potentially contribute to cancer onset.

2. OBJECTIVES

The primary objective of the study is to identify risk factors for cancer onset in the population affected by T2D.

Secondary objectives include:

1. Description of demographic, clinical, and first-line therapy characteristics of patients at the diagnosis of T2D.
2. Evaluation of risk factors for recurrence, presence of a second cancer unrelated to the first, and presence of both events in patients who have had cancer.
3. Investigating the correlation between patient characteristics and time to cancer onset.

3. MATERIALS AND METHODS

3.1 Study Design

The study follows a retrospective, monocentric cohort design, utilizing data sourced from the Smart Digital Clinic electronic medical record (Meteda Srl).

3.2 Participating Center

Endocrinology and Diabetology Outpatient Clinic of the SCDU of Novara, University of Eastern Piedmont. Responsible: Professor Flavia Prodam.

3.3 Subjects

All patients visited at the Endocrinology and Diabetology Outpatient Clinic of the AOU Maggiore della Carità in Novara for a first diagnosis of T2D between 1990 and 2010 will be included in the study.

3.3.1 Inclusion Criteria

- Adult age
- Diagnosis of T2D

3.3.2 Exclusion Criteria

- Diagnosis of tumor before the diagnosis of T2D
- Diagnosis of secondary diabetes due to other pathologies
- Diagnosis of secondary diabetes due to other medications
- Diagnosis of secondary diabetes due to surgical interventions

3.4 Intervention

No intervention of any kind.

3.5 Study Duration

24 months.

3.6 Follow-up and Events of Interest

Patients included in the study will be followed from the date of diagnosis of T2D until the date of the last available visit.

During the follow-up, for all included patients, the year of onset of the first cancer occurring after the diagnosis of diabetes and the type of cancer will be recorded. From this information, it will be possible to calculate the time elapsed between the diagnosis of diabetes and the onset of the tumor (measured in years).

For patients who have developed cancer, the following will also be recorded:

- Cancer recurrence (year of recurrence onset)
- Diagnosis of a second cancer unrelated to the primary cancer (year of onset, type of cancer)

Cancer diagnosis will be identified through the extraction system from the Smart Digital Clinic electronic medical record (Meteda Srl), using relevant keywords in the cancer domain, identified in the medical history section. The keywords will include: metastasis, adenocarcinoma, carcinoma, neoplasm, secondary, sarcoma, cancer, adenoma, lymphoma, leukemia, glioma, glioblastoma, basal cell carcinoma, squamous cell carcinoma, melanoma, mesothelioma, chordoma, anaplastic, differentiated, undifferentiated, meningioma, multiple myeloma, small cell carcinoma, thymoma, craniopharyngioma, neuroendocrine, LH, NHL, K, GIST, HCC, and NET.

3.7 Data Collection

For each patient, the following variables will be extracted from the Smart Digital Clinic electronic medical record extraction system, when possible:

- Gender and age;
- Smoking habit and alcohol consumption (units per day);
- Weight and body mass index (BMI) at the first (T0) and last visit (T1);
- Date of diagnosis of T2D;
- Date of the first visit;
- Treatment for T2D at T0 and T1;
- Levels of glycated hemoglobin (HbA1c) at T0 and T1, average HbA1c, and average fasting blood glucose;
- Creatinine clearance at T0 and T1;
- Liver enzymes at T0 and T1: alanine aminotransferase (ALT) and aspartate aminotransferase (AST);
- Lipid profile at T0 and T1: low-density lipoprotein (LDL-c) and triglycerides (TG);
- Complications of T2D;
- Treatment for the cancer;
- Family history of cancers.

The diagnosis of T2D is confirmed at diabetic centers or, in some cases, by general practitioners who refer patients to specialized diabetic centers. The accuracy of the diagnosis will be confirmed by cross-referencing with the Piedmont Diabetic Registry (PDR) and involving a second person to ensure precise assessment.

Glycated hemoglobin will be considered high if its values exceed or equal 8%, indicating glycemic decompensation, while it will be considered low if it presents values below 8%.

BMI categories will be divided into underweight (BMI <18.5 kg/m2), normal weight (BMI 18.5 – 25 kg/m2), and overweight (BMI >25 kg/m2).

Regarding the treatment of T2D, participants will be categorized and grouped for statistical purposes into the following classes:

- Dietary therapy;
- Metformin/Acarbose;
- Sulfonylurea;
- Metformin + GLP1/DPP4 inhibitors (DPP4i) or only GLP1 or only DPP4i;
- Metformin + SGLT2 inhibitors (SGLT2i) or only SGLT2i;
- Basal insulin + GLP1/DPP4i +/- Metformin;
- Basal insulin +/- Metformin +/- SGLT2i;
- Basal bolus insulin;
- Basal bolus insulin +/- Metformin +/- SGLT2i.

Furthermore, complications of metabolic pathology will be extracted from the dedicated section of the program, where they are systematically recorded and organized, and their diagnosis is conducted in accordance with appropriate guidelines. These complications will be divided into the following categories: vasculopathy, neuropathy, hypertension, heart disease, renal failure, and retinopathy.

Finally, regarding cancer, treatment will be categorized into three classes: chemotherapy, surgery, and radiotherapy. Additionally, cancer types will be divided to ensure greater homogeneity among groups, including the nervous system, head and neck, thorax, gastrointestinal, gynecological, urinary tract, male genital system, skin, blood, breast, soft tissues, endocrine glands, and neuroendocrine cancers.

Such information will be collected at the diabetes diagnosis visit and the last visit before cancer onset for subjects experiencing the event, and at the last available visit for the remaining subjects.

3.8 Sample Size

Assuming a type I error of 0.05, a power of 80%, a prevalence of subjects with high glycated hemoglobin levels of 13%, and a correlation between covariates in the model of 0.1, it will be necessary to identify 779 cancer cases to highlight a Hazard ratio of 1.2 for the association between glycated hemoglobin and cancer onset.

3.9 Statistical Analysis

Descriptive statistics will be calculated to summarize patient information.

Categorical variables will be reported as absolute frequencies and percentages, while numerical variables will be reported as mean and standard deviation or median (Q1-Q3) if not distributed according to a normal random variable according to the Shapiro-Wilk test and observation of the QQ plot.

Univariate and multivariate Cox proportional hazards regression models will be used to estimate hazard ratios (HRs) and corresponding 95% confidence intervals (95% CI) for the association between some risk factors, including particularly glycemic level, and cancer onset. The stepwise method will be used to select variables to include in the model. For this analysis, time to event will be calculated as the number of years between cancer onset and diabetes diagnosis for subjects who develop the cancer, and as the number of years between the last available visit and the date of diabetes diagnosis. The possibility of conducting an analysis separately for cancer type or considering specific cancer types based on the number of observed events will be evaluated. The same models will be used to estimate the association with the risk of recurrence, second cancer, or both in patients who have developed cancer. In this case, time to event will be calculated considering the difference in years between recurrence onset, second cancer, or last visit and the onset of the first cancer.

Linear regression models will be used to evaluate the relationship between variables measured at diagnosis and time to cancer onset. In case the assumptions of the linear model are not met (normality of residuals and homoscedasticity), the Spearman correlation coefficient will be calculated to measure the correlation between numerical variables and time to tumor onset, while the Mann-Whitney or Kruskall-Wallis tests will be used when variables measured at baseline are categorical.

The significance level will be set at 0.05, and all tests performed will be two-tailed. Finally, the analyses will be conducted using SAS software (version 9.4; SAS Institute, Cary, NC, USA).

4. EXPECTED RESULTS

Through this research, we aim to obtain new information about the study population in order to better understand the possible correlation between the two pathologies. This will enable us to identify risk factors associated with metabolic pathology that may influence the development time of cancer, as well as to identify those that may contribute to carcinogenesis itself.

This knowledge will allow us to define the role of hyperglycemia, obesity, and other risk factors or behaviors in the context of cancers in order to act with appropriate prevention strategies, considering cancer as one of the possible complications of T2D.

5. ETHICAL ASPECTS

The study has been designed and will be conducted in accordance with international and national ethical standards for biomedical research involving human subjects, particularly:

- Ethical principles for medical research involving human subjects (Declaration of Helsinki - World Medical Association, current version);
- Good Clinical Practice (ICH/GCP) guidelines of the European Union;
- Convention on Human Rights and Biomedicine (Oviedo Convention of 04/04/1997);
- Italian codes of ethics for healthcare professions and specific national legislation regarding clinical studies.

The study is observational compared to what is done in normal clinical practice.

5.1 Informed Consent Management

An informative sheet is available explaining the type of study, the purposes, procedures, sample and data collection, potential benefits of the research, the absence of specific risks, as it is an observational study. This information will be presented to interested individuals, who will have the freedom to give consent or to withdraw it from the ongoing study.

5.2 Management of Sensitive Data

The following European and national regulations are taken into account:

- Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 and subsequent amendments published in the Official Journal of the European Union 127 of 23 May 2018 (GDPR);
- Legislative Decree 30 June 2003, n. 196 (Personal Data Protection Code), as amended by Legislative Decree 10 August 2018, n. 101 containing "Provisions for the adaptation of national legislation to the provisions of Regulation (EU) 2016/679";
- Article 8 of Legislative Decree 101/2018 of 19 September 2018, for the processing of data concerning minors under the age of 16, for the expression of informed consent. The Legislative Decree harmonizes the Privacy Code (Legislative Decree 196/2003) with EU Regulation 2016/679.

5.3 Data Processing Methods

The study involves the collection of information regarding lifestyles and pathologies of the subjects involved. The personal/sensitive data collected will be immediately pseudonymized by randomly assigning alphanumeric anonymous codes, which will be known and held only by the responsible

research experimenter. Therefore, only the responsible research experimenter will be able to link the information and data obtained from their analysis to a specific subject.

Personal data will be processed for the purposes outlined in the project, according to the principles of lawfulness, fairness, transparency, purpose limitation, data minimization, and accuracy (Art. 5 GDPR) in both paper and electronic form by authorized data processors. The availability, management, access, storage, and usability of the data are guaranteed by the adoption of technical and organizational measures to ensure adequate levels of security (Arts. 25 and 32 GDPR).

The data obtained from the research will not be retained beyond the time necessary for data analysis. Only researchers involved in the study will have access to the data. The research results will be made public or used for communications/scientific publications, only in anonymous and aggregated form.

The data controller is the A.O.U. Maggiore della Carità in Novara.

6. COSTS

The funds available to the Investigator derive from the NODES project, which has received funding from the MUR - M4C2 1.5 of the PNRR with grant agreement number ECS00000036.

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