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# Levels of interleukin-2 in patients with colon cancer and diabetes type 2

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## Abstract

**Introduction and objectives.** The risk of development colon cancer (CC) is increased significantly among patients with the type 2 diabetes (T2DM). A mechanism responsible for the higher prevalence of CC among diabetic patients may be associated with the immunity system. The aim of this study is to point out the differences in the immunity state in terms of interleukin 2 level among patients with T2DM suffering from CC, and patients without these diseases.

**Materials and methods.** 79 patients were included the tests, divided into 4 groups: Group 1–23 people with T2DM, Group 2–23 people with large intestine CC, Group 3–10 people with large CC and T2DM, and Group 4–23 people without T2DM or CC. Each patient had a colonoscopy and those with cancer were confirmed in a histopathological examination. Laboratory measurements included fasting glucose, insulin, C-peptide. The concentration of interleukin-2 in serum was determined with the immunoenzymatic (ELISA) method.

**Results.** The results obtained showed that in patients with T2DM and CC the concentration of interleukin-2 was statistically higher than in the other groups.(4.21±1.61 pg/ml vs. Group 1 -1.64±0.44 pg/ml, Group 2–1.54±0.21 pg/ml, and Group 4–1.70±0.36 pg/ml; $p<0.05$). Insulin levels, C-peptide and HOMA-IR did not differ significantly between groups, but a tendency was observed to higher values of HOMA-IR and insulin levels in the groups with T2DM alone and T2DM with concomitant CC.

**Conclusions.** The data show differences in the immunity state of patients with T2DM and CC, compared with people without those two diseases. Elevated level of interleukin 2 found in this group, after confirmation in other studies with more patients, could be used as a marker of an increased risk of CC in people with T2DM.

## Key words

diabetes type 2, colon cancer, interleukin-2

## INTRODUCTION

In Poland, the number of patients suffering from type 2 diabetes (T2DM) exceeds 3 million. It is estimated that this group includes about one-third patients with undiagnosed diabetes. Taking into account the results of the NATPOL study (Nadciśnienie Tętnicze w Polsce – High Blood Pressure in Poland), the prevalence of T2DM increases in the Polish population by 2.5% annually [1]. Colon cancer (CC) is one of the most common malignancies worldwide. It is the third most common cancer in both genders, and the second as a reason of cancer-related death worldwide. It is estimated that the lifetime risk of development CC is about 6% and most patients die from the spread of metastasis [2, 3]. A common coexistence of T2DM and cancer, especially glandular tumours (including CC) has been observed [3, 4].

A very relevant mechanism which can influence the increased occurrence of cancer together with diabetes is the disturbed function of the immune system. One of the elements of the immunity system is cytokines. Studies show the influence of interleukin-2 on the proliferation of T regulatory lymphocytes, which take part in the development of cancer antigen tolerance. It facilitates the development of cancer, which was shown in research concerning cancer of large intestine [5, 6].

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## OBJECTIVE

The purpose of this study was to demonstrate the differences in the immune system in terms to the concentration of interleukin 2 in a group of patients with T2DM with concomitant CC, compared with patients without CC and without T2DM. The studies was additionally conducted to identify possible relationships between the level of interleukin-2 and the risk of developing CC in patients with T2DM.

## MATERIALS AND METHOD

The study was performed in the Department of Internal Diseases, Diabetology and Endocrinology and the Department of General and Vascular Surgery at the Medical University in Warsaw, and the Clinic of Metabolic Diseases and Gastroenterology Institute of Food and Nutrition, also in Warsaw. All persons participating in the study were informed about the purposes and the schedule of experiment, and also about the rules for their safety. Each patient was included in

the study after obtaining conscious written consent. Persons authorized for individual groups were subjected to an internal study, with a detailed account of the interviews collected in the form of survey data on demographic, environmental, clinical and family history. Qualifying tests were conducted under the protocol approved by the Commission of Bioethics at the Medical University in Warsaw. All patients had a colonoscopy performed. In the case of cancer, a histopathological study and computed tomography were performed. Patients with inflammatory bowel disease were excluded from in the study.

The study included 79 patients who were divided into 4 groups: Group 1 – 23 patients with T2DM, Group 2 – 23 patients with CC, Group 3 – 10 patients with T2DM and CC, and Group 4 – 23 persons as the control, without T2DM and cancer. The study did not include patients with any early diagnosed cancer disease, receiving systemic corticosteroids, women who were pregnant and those during lactation, people who are dependent on psychotropic substances, alcohol and drugs. The patients with T2DM were treated with different medication which included metformin, sulfonylurea, inhibitor DPP-4 (dipeptidyl peptidase-4 inhibitor) and insulin.

**Laboratory methods.** Laboratory measurements were performed in the research laboratory of the Clinic of Internal Diseases, Diabetology and Endocrinology at the Medical University in Warsaw, and in the laboratory of the Mazovian Hospital Bródnowski in Warsaw. The laboratory studies included the following examinations: fasting plasma glucose (FPG) in the plasma of venous blood by the enzymatic method implemented with glucose oxidase and marking of H2O2, insulin by the radioimmunologic method, C-peptyd. HOMA-IR (homeostatic model assessment – insulin resistance) was calculated by the formula: concentration of fasting insulin (mU/l) x fasting glucose level (mmol/l)/22.5. The level of transaminases and creatinine in serum was investigated. Patients with renal insufficiency with creatinine above 2 mg/dl and transaminase levels above 3 times the upper limit of normal were excluded from the study. Identification of interleukin-2 was made at the Department of Clinical Immunology, Institute of Transplantology at the Medical University in Warsaw. 5 ml of peripheral blood from the cubital vein was taken into test tubes, without anticoagulants. The blood was then subject to centrifugation at a speed of 2,000 rpm for 10 minutes. The obtained supernatant was portioned in properly marked containers and next was frozen at -70 °C. The concentration of interleukin-2 was analyzed by the immunoenzymatic method ELISA (enzyme linked immunosorbent assay) with ready-to-use jet kits Human IL-2 ELISA (DIACLONE Research, France) in binary reps.

**Statistical methods.** Statistical significance was evaluated using chi-square test (gender composition of groups) or one-way analysis of variance (ANOVA), followed by a *post hoc* Fisher's test (all the other parameters under study). Differences were considered statistically significant when the p value was <0.05. All statistical analyses were performed using Statistica 10.

## RESULTS

The results of the studied groups did not differ significantly among themselves in terms of gender (p=0.1977 by Chi–square) and age (p-value 0.2145).There was no statistically significant difference between groups in BMI (p-value 0.708) (Tab. 1).

**Table 1.** Parameters of patients in the four groups: Group 1- patients with diabetes mellitus type 2, Group 2 – patients with colon cancer, Group 3 – patients with colon cancer and diabetes mellitus type 2, Group 4 – control, people without diabetes and without colon cancer

| Parameter | Group 1 T2DM | Group 2 CC | Group 3 T2DM+CC | Group 4 Control | P |
|---|---|---|---|---|---|
| No. of patients | 23 | 23 | 10 | 23 | |
| Female/Male | 15/8 | 11/12 | 6/4 | 18/5 | 0.1977 |
| Average agee, years | 70.74 | 67.43 | 71.50 | 66.26 | 0.2145 |
| Age standard deviation | 6.55 | 11.00 | 9.03 | 7.78 | |
| BMI average, kg/m$^2$ | 28.72 | 24.95 | 27.83 | 27.46 | 0.708 |
| BMI standard deviation | 5.45 | 4.61 | 5.55 | 4.16 | |
| % of smoking patients | 21.7% | 26% | 20% | 21.7% | |

In the group of patients with coexistence of T2DM and CC, a statistically higher concentrations of interleukin-2 (4.21±1.61 pg/ml) was observed than in the other groups (group 1 -1.64±0.44 pg/ml, group 2 – 1.54±0.21 pg/ml, group 4 – 1.70±0.36 pg/ml. (Fig. 1).

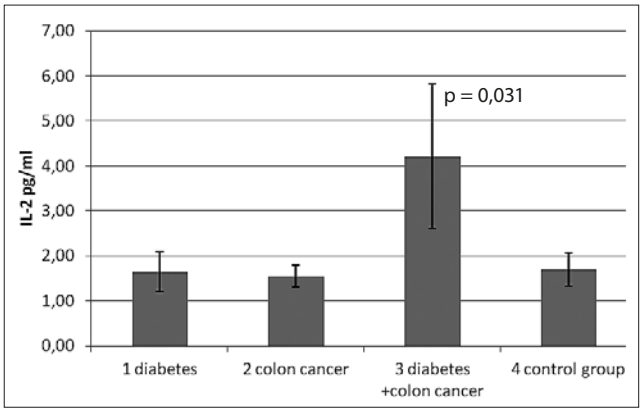


**Figure 1.** Level of interleukin-2 in four groups: Group 1- patients with diabetes mellitus type 2; Group 2 – patients with colon cancer; Group 3 – patients with colon cancer and diabetes mellitus type 2; Group 4 – control, people without diabetes and without colon cancer

There was no statistically significant difference between groups in the levels of serum insulin, C-peptide or HOMA-IR; however, higher values of HOMA-IR and insulin levels were noted in the groups with T2DM alone and T2DM with concomitant CC. Also in these groups there was observed a statistically higher level of FPG. (Tab. 2).

**Table 2.** Studied parameters in the four groups: Group 1 – patients with diabetes mellitus type 2, Group 2 – patients with colon cancer, Group 3 – patients with colon cancer and diabetes mellitus type 2, Group 4 – control, people without diabetes and without colon cancer

| Parameter | Group 1 T2DM | Group 2 CC | Group 3 T2DM+CC | Group 4 Control | P |
|---|---|---|---|---|---|
| IL -2 average, pg/ml | 1.64 | 1.54 | 4.21 | 1.70 | 0.0310 |
| IL- 2 standard terror | 0.44 | 0.24 | 1.61 | 0.36 | |
| Fasting insulin average, uIU/ml | 12.81 | 8.91 | 17.09 | 9.01 | 0.2389 |
| Fasting insulin standard error | 3.64 | 1.59 | 4.81 | 1.27 | |
| C-peptyd average, ng/ml | 2.88 | 2.44 | 3.38 | 2.51 | 0.5117 |
| C-peptyd standard error | 0.53 | 0.30 | 0.51 | 0.19 | |
| FPG average, mmol/l | 7.08 | 4.95 | 6.36 | 5.20 | 0.0001 |
| FPG standard error | 0.43 | 0.13 | 0.48 | 0.21 | |
| HOMA IR average | 4.41 | 1.99 | 4.82 | 2.19 | 0.0860 |
| HOMA IR standard error | 1.36 | 0.34 | 1.51 | 0.35 | |
| CRP average, mg/l | 7.80 | 17.93 | 10.35 | 13.38 | 0.4745 |
| CRP standard error | 2.79 | 6.07 | 2.72 | 5.34 | |

## DISCUSSION

T2DM is often associated with cancer, and the risk of CC development among T2DM patients is about 30% higher than among people not suffering from diabetes. Statistically, there is no significant difference in the occurrence of CC between men and women with T2DM [7]. There are numerous epidemiological studies which confirm this observation. Taiwanese research showed a higher risk of development of breast, colon, liver, pancreas, lung and prostate tumours in patients with T2DM [4]. The meta analysis of Yuhara et al. showed that T2DM is an independent factor in the development of large intestine and rectal cancer. This dependency does not change after additional evaluation of such factors as smoking, obesity and lack of physical exercise. The risk of CC development among T2DM patients was greater than the risk of the development of rectal cancer. These differences are possibly because different sections of the large intestine (proximal, distal and rectum) have different embryological origins. In the presented study, T2DM was related with the higher risk of CC development among men and women, although in terms of rectal cancer, this dependency was noted only among men [8].

He et al., in a prospective, multiethnic, cohort research that evaluated over 200,000 people of Caucasian, Afro-American, Japanese, native Hawaiian and Latino origin, confirmed a higher risk of CC development among people with diabetes, compared with persons without diabetes in all groups, with the exception of native Hawaiian people [9].

On the basis of meta analysis of controlled, prospective, multiethnic, cohort research published in 2007, de Bruijn et al. deduced that T2DM is a risk factor of breast and CC, as well being a risk factor of dying from these illnesses [10].

A few pathogenic mechanisms leading to the development of tumours in people with T2DM are considered. Taking into account the effects of hyperglycaemia, hyperinsulinaemia and insulin resistance, a link was observed between overweight and obesity, and chronic inflammation and impaired immune system [2, 11, 12]. The European Prospective Investigation into Cancer and Nutrition Study (EPIC) showed that elevated levels of C-peptide in the blood plasma as a marker hyperinsulinaemia positively correlated with the risk of development colon and rectum cancer. Also, a high level of C-peptide and low level of IGFBP-1 (insulin-like growth factor-binding protein 1) was associated with a higher risk of death after previous surgery for colon and rectum cancer. Insulin is an anabolic hormone which intensifies lipogenesis, DNA, protein and glycogen synthesis but slows down lipolysis, gluconeogenesis and glycogenolysis. With high concentrations of insulin, mitogenic and anti-apoptotic effects start to intensify. Insulin stimulates the growth and differentiation of cells. Connecting with the specific receptors IR (insulin receptor) and IGF-1-R (insulin-like growth factor 1 receptor) and working through the pathways of MAPk (mitogen-activated protein kinase) and PI3K (phosphoinositide 3-kinase), insulin sends mitogenic signals into the cell`s nucleus, increases the synthesis of proteins and fatty acids, inhibits apoptosis. In addition, insulin inhibits proteins that bind IGF (insulin-like growth factor-binding proteins) which results in increased activity of IGF-1, IGF-1 receptors. The IGF system is an important factor of growth associated with cancerogenesis [8]. Cancer cells have an increased receptor expression for insulin and IGF-1, which leads to escalation of insulin influence in the cancer expansion.

T2DM is often associated with abdominal obesity which involve disorders in the fatty tissue, such as abnormalities of transmission of intracellular signals, insulin resistance, and activation of adypocytes that produce pro-inflammatory adipokins. Adipokins stimulate other adypocytes and macrophages of fat tissue to produce pro-inflammatory factors. Many studies have revealed increased parameters, such as TNF-alpha (tumour necrosis factor- alpha), interleukin-2, INF-γ (interferon gamma), CRP (C-reactive protein) in serum in people with type 2 diabetes [13, 14, 15]. Therefore, it results in chronic inflammation in the adipose tissue and beyond. The correlation between obesity, which is often associated with T2DM, and the development of colon tumour may be associated with chronic inflammation [16, 17, 18]. Pro-inflammatory cytokines produced and secreted through the fatty tissue have a potentially neoplasmatic effect [19, 20]. The excess of substances, such as interleukin-6, adiponectin leptin, TNF-alpha, PAI-1 (plasminogen activator inhibitor-1), and MCP-1 (monocyte chemoattractant protein-1), may play an important role in cancer cell transformation or their development [21, 22]. Disorders of cytokines and their functions are inflammatory factors directly or indirectly affecting the functioning of the immune system. Cytokines, which may affect the development of cancer, include interleukin-2, interleukin-6, interleukin-10, TNFα, and interferon γ [23, 24].

Interleukin-2 is produced by Th1 lymphocytes antigen recognizing and in smaller number of cytotoxic T lymphocytes. Expression of interleukin-2 can be increased more than 1,000 times after the of lymphocyte. The main directions of action of interleukin-2 is proliferation and activation of regulatory T lymphocytes. IL-2 is also involved in the activation of cytotoxic T lymphocytes (Tc lymphocytes), and differentiation of T lymphocytes towards cytotoxic lymphocytes. Tc cells take part in the destruction

of cancer cells [25]. It has been confirmed that IL-2 enhances the antitumour effect of CD8+ cells [26]. The binding of IL-2 to regulatory T cells reduces the availability of cytokine to the effector T cells. IL-2 increases proliferation, activation and cytotoxicity of NK cells (natural killer cells) and B lymphocytes [25].

It is interesting that interleukin-2 affects the proliferation of regulatory T-cells which participate in the development of tolerance to antigens associated with cancer. As shown in studies on populations of the Far East, there are dependencies between activated regulatory T-lymphocytes and the progression of colon cancer. Activated regulatory T lymphocytes in colon cancer tissue were correlated with cancer metastasis, and impacted negatively on the cancer immune system of patients [5, 6]. Studies conducted by Svensson H, et al. on subpopulations of lymphocytes which infiltrate the cancer tissue and normal mucosa in patients with colon cancer, showed that regulatory T lymphocytes accumulate in the tissues changed by cancer, while the activated Th1 lymphocytes are less represented. Changed proportions of the composition of the different types of lymphocytes in CC can probably have an impact on reducing the capabilities of the immune system to properly attack a cancer [27, 28].

## RESULTS

The results of the presented study indicate differences in the immune system in patients with T2DM with coexistence of CC, compared with people without T2DM and without CC. A statistically higher concentration of interleukin-2 was observed in the group of patients with T2DM and CC, than in the groups without diabetes or large intestine cancer. The higher level of interleukin-2 can be potentially connected with an increased risk of CC.

The concentrations of serum insulin, C-peptide and HOMA-IR did not statistically significantly differ among the groups, although higher values of HOMA-IR and insulin levels were noted in the groups with T2DM alone and T2DM-associated CC.

## CONCLUSIONS

Elevated concentrations of interleukin-2 identified in the group of patients with T2DM and CC, after confirmation in other studies on a larger number of patients, can be used as a marker for increased risk of developing CC in people with T2DM. The current findings can contribute to the procedures in the screening of colon cancer in people with T2DM. Detailed recognition of the risk factors for colon cancer, as well as differences in immunological studies in individuals with coexistence of T2DM, could become the basis for strategy for the prevention of this cancer, and may help in the selection of groups of patients among people with T2DM, especially those prone to development of cancer.

The coexistence of T2DM and CC is characterized by the increased level of serum interleukin-2. Interleukin-2 can potentially have an influence on the development of cancer and progress of malignancy. It seems that patients with T2DM and increased level of interleukin-2 should be taken into special oncological care.

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