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Evaluation of Interferon-Gamma in Patients with Type 2 Diabetes and Colorectal Cancer

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Abstract

Introduction and objectives: Multiple studies suggest a common coexistence of type 2 diabetes mellitus (T2DM) and cancer, especially colorectal cancer (CC). One of the most important mechanism that may have an impact on the increased incidence of cancer in diabetes is deregulation in the immune system. Interferon gamma (IFNγ), a cytokine critical for anti-tumor immunity, enhances cytotoxic activity of Tc lymphocytes, natural killer (NK) cells, activates macrophages, affects the apoptosis and induces the production of other cytokines such as IL-2, IL-6. The aim of this study was to evaluate the concentration of IFNγ in patients with T2DM with accompanying colorectal cancer compared to patients with T2DM or colorectal cancer separately.

Materials and methods: This study was performed in Department of Internal Diseases, Diabetology and Endocrinology and Department of General and Vascular Surgery, Medical University of Warsaw, and the Clinic of Metabolic Diseases and Gastroenterology Institute of Food and Nutrition in Warsaw. In this study 79 patients were enrolled. They have been divided into 4 groups: group 1 (23 subjects) with T2DM, group 2 (23 subjects) with CC, group 3 (10 subjects) – with CC and T2DM, and group 4 (23 subjects) without T2DM or CC. All patients had a colonoscopy performed. In the case of cancer there was done histopathological study. Laboratory measurements included fasting glucose, insulin, C-peptide, HbA1c, lipidogram. The concentration of IFNγ in serum was determined with the immunoenzymatic (ELISA) method.

Results: IFNγ level in patients from group 1 (T2DM) was 3.13 ± 0.92 pg/ml, group 2 (CC) –2.73 ± 0.91 pg/ml, group 3 (T2DM and CC) – 2.46 ± 0.98 pg/ml and group 4 (control) –5.02 ± 1.43 pg/ml; $p < 0.05$. There was no statistically significant difference in the concentration of IFNγ in patients with T2DM and CC compared to other subjects. However, it has been demonstrated that level of IFNγ in the control group and the group of patients with T2DM without CC was higher than in the other two groups. There was no statistically significant difference between the groups in levels of insulin, C-peptide and HOMA-IR.

Conclusions: The concentration of IFNγ did not differ significantly between all studied groups of patients. A better understanding of the role of IFNγ in T2DM and CC will contribute to identification of risk factors, more precise diagnosis and treatment of both diseases. Future studies are needed to confirm the validity of these observations.

Keywords: Diabetes type 2; Colorectal cancer; Interferon γ

Introduction

The number of patients suffering from type 2 diabetes is increasing. According to data from the International Diabetes Federation (IDF-the International Diabetes Federation) from 01.12.2015 it is estimated that 415 million people in the world have diabetes. About half of these people are undiagnosed. It is expected by the IDF that the number of people with diabetes by 2040 will reach 642 million people [1].

In Poland, taking into account the results of the study NATPOL, the number of diabetics is estimated at more than 3 million people, including about 1/3 of people with previously undiagnosed diabetes [2].

A relevant worldwide problem is also a growing number of cases of cancer. It is estimated that the number of cancer patients will increase from 12,700,000 in 2008 year to 22,200,000 in 2030 year [3]. Colorectal cancer (CC) is one of the most common cancers in the world. It is the third in frequency in men and women and the second as a cause of cancer mortality in the world [4,5].

Multiple studies suggest a common coexistence of T2DM and cancer, especially CC [5,6]. In 2010, the American Diabetes Association (ADA) and the American Cancer Society (ASC) announced a joint consensus for diabetes and cancer, relationship of diabetes with cancer and its development, common risk factors for diabetes and cancer, molecular mechanisms linking diabetes and cancer and the impact of hypoglycemic therapy on the risk of cancer or cancer prognosis. They concluded that diabetes is associated with increased risk of cancer such as cancer of the liver, pancreas, endometrium, colon, breast, gallbladder. It was confirmed that a healthy diet, physical activity and normal body weight reduces the risk of developing diabetes and some cancers, as well as improve the prognosis.

The Japan meta-analysis of 8 cohort studies has assessed more than 330,000 people over 10 years. It has been shown that diabetes is

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associated with an increased risk of all cancers in both men and women - HR (hazard ratio) 1.19, 95% CI 1,12-1,27 and HR 1.19, 95% CI 1.07 -1.31 respectively. It has been listed in patients suffering from diabetes an increased risk of developing colorectal cancer (HR 1.40, 95% CI 1,19-1,64), liver cancer (HR 1.97, 95% CI 1,65-2,36) pancreatic cancer (HR 1.85, 95% CI 1,46-2,34). Also a tendency to an increased risk of endometrial cancer and gall bladder cancer in diabetics was observed [7].

Meta-analysis carried out by Larsson et al. [8] revealed that the incidence of colorectal cancer in patients with type 2 diabetes is higher by approximately 30% compared to those without diabetes. There is no statistically significant difference in the risk of colorectal cancer among men and women with type 2 diabetes: in women - summary RR 1.33, 95% CI=1.23-1.44, men - summary RR 1.29, 95% CI=1.15- 1.44.

He et al. [9] in a prospective multiethnic cohort study, which evaluated more than 200 thousand people (Caucasians, African Americans, Japanese, Native Hawaiians, Latinos) confirmed the significantly higher risk of colorectal cancer among patients with diabetes compared with people without diabetes (RR = 1.19, 95% CI = 1.09-1.29) in all ethnic groups except for a group of Native Hawaiians. It was observed for cancer localized in different parts of the colon (ascending colon, transverse colon, descending colon), tumors of various stages - locally and more advanced. For rectal cancer it was observed an insignificant positive correlation between diabetes and risk of this cancer. Latinos were the ethnic group where the strong correlation between rectal cancer and diabetes was observed.

De Bruijn et al. [10] based on meta-analysis of controlled prospective cohort studies published after 2007 concluded that type 2 diabetes is a risk factor for colon cancer as well as breast and increased mortality due to these cancers. HR colorectal cancer was 1.26 (1.14 to 1.40), HR for mortality from colorectal cancer was 1.30 (1.15 to 1.47) for patients with diabetes compared to those without diabetes.

It is contemplated several pathogenic mechanisms leading to tumor growth in patients with type 2 diabetes, which include the effects of hyperglycemia, hyperinsulinemia and insulin resistance, excess of fatty tissue, chronic inflammation and immune system abnormalities [4,11]. Moreover, abnormalities in the immune system functioning may have an impact on increased risk of cancer in diabetic patients.

The type of hypoglycemic treatment may also play a role in the development of cancer in patients with diabetes type 2 [5,6].

Natural killer (NK) cells are the cells of the elements of the immune system which are involved in the destruction of tumor cells. It was found that the higher activity of NK cells is associated with a lower risk of developing cancer [11]. It was revealed that in obese subjects with metabolic abnormalities the number and activity of NK cells is decreased in the comparison to obese persons without glucose metabolism disorders, who had more NK cells and NK cells were more active [12,13].

The other components of the immune system which directly or indirectly affect the tumor growth are cytokines. Cytokines act in different directions, one cytokine may affect various cells and induce various (different) reactions [14].

Interferons belong to the group of cytokines, which are involved in antiviral and anti-tumor resistance, apoptosis, cell cycle control. These proteins also mediate other cytokines.

Interferon γ (IFNγ) is produced by T cells and NK cells activated by IL-2, IL-12, IL-15, IL-18, IL-21. It is involved in the differentiation of Tc lymphocytes, B cells, enhances the cytotoxicity of Tc cells, NK cells, induces the production of other cytokines such as IL-2, IL-6, TNF-α. In addition, it increases the expression of MHC (major histocompatibility complex) class II. IFNγ is the strongest activator of macrophages, which recognize and phagocytose cancer cells and microorganisms [14].

There are no screening studies evaluating the association of colorectal cancer and type 2 diabetes. Understanding the risk factors for colorectal cancer, and differences in immunological studies with accompanying type 2 diabetes could lead to a strategy for the prevention of colon cancer and may help to isolate a subgroup of type 2 diabetic patients who are particularly vulnerable to this cancer and need in the first the order of execution of screening for colorectal cancer.

The aim of this study was to evaluate the concentration of IFNγ in patients with type 2 diabetes with accompanying colorectal cancer compared to patients with type 2 diabetes or colorectal cancer separately and detect possible relationship between the level of IFNγ and the risk of colorectal cancer in patients with type 2 diabetes.

Materials and Methods

This study was performed in Department of Internal Diseases, Diabetology and Endocrinology and Department of General and Vascular Surgery, Medical University of Warsaw, and the Clinic of Metabolic Diseases and Gastroenterology Institute of Food and Nutrition in Warsaw. All persons participating in the study received information about the purposes and the schedule of experiment. They were informed 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 the internal study, with a detailed account of the interviews collected in the form of survey data on demographic, environmental, clinical, family history. Qualifying tests were conducted under the protocol approved by the Commission of Bioethics at the Medical University of Warsaw (nr 189/2009). All patients had performed a colonoscopy. In the case of cancer there was done histopathological study. Patients with inflammatory bowel disease were not included in the study.

For the study included 79 patients who were divided into 4 groups - group 1 (23 patients – 15 women, 8 men, average age 70,74 years old) with T2DM, group 2 (23 patients – 11 women,12 men, average age 67,43 years old) with CC, group 3 (10 patients – 6 women, 4 men, average age 71,5 years old) with T2DM and CC, and 4 group (23 patients – 18 women, 5 men, average age 66,26 years old) control, without T2DM and cancer.

Patients from both group 1 (T2DM) and group 3 (T2DM and CC) didn`t differ in respect of treatment by metformin, sulfonylurea and insulin.

The study did not include patients with some cancer disease early diagnosed, receiving systemic corticosteroids, both women pregnant and during lactation, patients who are dependent on psychotropic substances, alcohol, drugs.

Laboratory methods

Laboratory measurements were performed in the research laboratory of Clinic of Internal Diseases, Diabetology and Endocrinology, Warsaw Medical University and in the laboratory Masovian Hospital Bródnowski in Warsaw. Laboratory studies included the following examinations: fasting plasma glucose (FPG) in the plasma of venous blood by enzymatic method implemented with glucose oxidase and

marking of H_2O_2, insulin by radioimmunologic method, C-peptyd. The HOMA –IR (homeostatic model assessment – insulin resistance) was calculated according to the formula - the concentration of fasting insulin (mU/l) x fasting glucose level (mmol/l)/22,5. The level of transaminases and creatinine in the 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. The concentration of IFNγ was measured at the Department of Clinical Immunology, Institute of Transplantology of Warsaw Medical University by the immunoenzymatic method ELISA (enzyme linked immunosorbent assay) with ready-to-use jet kits Human INFγ ELISA (DIACLONE Research, France) in binary reps.

Statistical methods

For comparison of the studied groups were calculated statistical parameters characterizing the variability of the evaluated features. In order to compare the significances of differences between averages a single agent analysis was conducted and groups were created using the Fisher method. Comparing the groups in terms of gender percentages was carried out using the chi-square test of independence. Analysis was carried out using the number of men and women in each group. Assumed the level of significance 0.05. For comparison age, it was used the same method as for other characteristics, that is, analysis of variance and multiple comparisons Fisher procedure. In all analysis the 0.05 significance level was assumed [15].

Results

The results of the studied groups did not differ significantly among themselves in terms of gender (p=0.1977 by Chi–square) (Table 1) and age (p-value 0.2145).

There was no statistically significant difference in the concentration of IFNγ in the groups of patients with T2DM with CC compared to subjects with T2DM or with CC. However he level of IFNγ in the control group and the group of patients with T2DM without CC was higher than in the other two groups (Figure 1, Table 2).

There was no statistically significant difference between the groups in levels of insulin, C-peptide and HOMA-IR. Whereas it was observed trend for higher HOMA -IR and insulin levels in groups of type 2 diabetes and type 2 diabetes associated with colon cancer. Also in these groups showed a statistically significant higher levels of fasting plasma glucose and glycosylated hemoglobin. Total cholesterol and LDL cholesterol was highest in the control group, which can probably be explained less frequent medical supervision and less stringent medical requirements in relation to patients without diabetes.

Discussion

Type 2 diabetes is often associated with cancer. The risk of developing colorectal cancer in patients with type 2 diabetes is about 30% higher than in people without diabetes [8].

One of the possible mechanism that may have impact on the increased incidence of cancer in diabetes is abnormal immune system.

Interferon γ (P 0,441)	Group 1	Group2	Group 3	Group 4
Mean ± standard error, pg/ml	3.13 ± 0.92	2.73 ± 0.91	2.46 ± 0.98	5.02 ± 1.43

Table 1: The level of interferon γ in four groups of patients : 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.

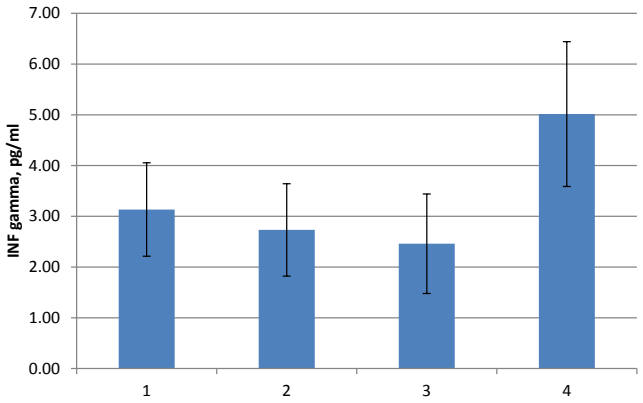

Figure 1: The level of interferon γ in four groups of patients : group 1- patients with diabetes mellitus type 2, group 2 – patients with colon cancer, group 3 – patients with colon cancer and diabetes mellitus type 2, group 4 - control, people without diabetes and without colon cancer.

Parameter	Group 1 T2DM	Group2 CC	Group 3 T2DM+CC	Group 4 Control	Significance level
Mean Fasting insulin ± standard error , uIU/ml	12.81 ± 3.64	8.91 ± 1.59	17.09 ± 4.81	9.01 ± 1.27	0.2389
Mean C-peptyd ± standard error, ng/ml	2.88 ± 0,53	2.44 ± 0.30	3.38 ± 0.51	2.51 ± 0.19	0.5117
Mean FPG ± standard error, mmol/l	7.08 ± 0.43	4.95 ± 0.13	6.36 ± 0.48	5.20 ± 0.21	0.0000
Mean HOMA IR ± standard error	4.41 ± 1.36	1.99 ± 0.34	4.82 ± 1.51	2.19 ± 0.35	0.0860
Mean Hba1c ± standard error,%	7.22 ± 0.27	5.76 ± 0.09	6.46 ± 0.30	5.89 ± 0.13	0.0000
Mean Total cholesterol ± standard error, mmol/l	168.17 ± 7.21	180.43 ± 8.42	164.72 ± 13.08	205.43 ± 8.27	0.0045
Mean LDL-cholesterol ± standard error, mmol/l	98.48 ± 6.01	114.26 ± 7.61	87.00 ± 8.55	133.65 ± 6.54	0.0003

Table 2: Studied parameters 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, 4 group - control, people without diabetes and without colon cancer.

IFNγ is a soluble cytokine which directly or indirectly affect the tumor growth. IFNγ enhances the presentation of tumor associated antigens to T cells, activates macrophages, impacts to inhibition of proliferation and stimulation of differentiation of tumor cells. It has cytotoxic activity, inhibits the formation of blood vessels and increases the expression of MHC molecules [14,16].

Wang et al. maintains the thesis that INFγ and activation of INFGR1(Interferon gamma receptor 1) are factors limiting the development of colon cancer [17].

Gerber et al. [18] reports that INFγ is important in getting anti-cancer effect with the use of radiation therapy in colorectal cancer. A

significant reduction in the level of IFN γ by eliminating CD8 (+) T cells resulted in a reduction in the effect of radiotherapy.

Mori et al. [19] evaluated the antiproliferative activity of IFNγ affecting the activation of the receptor R2. Giving rise to the expression of the receptor INFGR2 in cell lines of colorectal cancer, received the increasing of an antiproliferative effect of IFNγ and induction of apoptosis of colon cancer cells.

INFγ action has proapoptotic effect on tumor cells of the colon (LRCCs- cancer label-retaining cells). In these cells, there is a big number of INFγ receptors. Ni et al. [20] suggested that the using of INFγ might be used in the treatment of cancer.

These evidences suggest an important role of IFNγ in the development of cancer. INFγ is only one of elements of INF signaling pathway. Some genetic variation of IFN signaling pathway, including IFNγ and its receptors, IRFs (Interferon Regulatory Factors) are associated with the risk of developing colorectal cancer [16]. Another aspect of the IFNγ action is the weakening of the activity of insulin, thus it leads to insulin resistance, which is considered as one of the possible mechanisms of increased carcinogenesis in diabetes.

In our laboratory tests, there was no statistically significant difference in the level of IFNγ in serum blood in groups of patients with colorectal cancer with type 2 diabetes and without diabetes. There was a trend for higher levels of IFNγ in the control group and group of patients with type 2 diabetes without colorectal cancer. This data could suggest that the level of IFNγ and its protective action in patients with colorectal cancer are smaller than in ones without cancer.

Although insulin resistance with elevated insulin level is considered as one of the factors of increased risk of developing cancer, in our study we showed no statistically significant difference in the insulin level, HOMA IR between the groups. The high level of insulin could be observed in the beginning of T2DM, later the insulin concentration gradually decreases. It could be a one reason why there was not a elevated insulin level in group 1 with T2DM and group 3 with T2DM and CC.

Conclusions

The concentration of IFNγ did not differ significantly between all studied groups of patients. A better understanding of the role of IFNγ in T2DM and CC will contribute to identification of risk factors, more precise diagnosis and treatment of both diseases. Future studies are needed to confirm the validity of these observations.

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