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The Evaluation of Cellular and Humoral Immunity Characteristics in Gastrointestinal and Hepatocellular Cancers

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ABSTRACT

BACKGROUND: *Gastrointestinal (GI) cancers (Ca) are a global health problem leading to high mortality around the world. Human organism presents a perfect defense against cancers by immune system. In this study, we aimed to evaluate the characteristics of the humoral and cellular immune systems in GI Ca patients.*

MATERIALS AND METHODS: *Cellular and humoral immune parameters (CD3, CD4, CD8, CD16/CD56 and CD19 lymphocytes) were investigated in the peripheral blood of advanced 198 GI and hepatocellular cancer (HCC) patients by flow cytometry.*

RESULTS: *CD8 lymphocyte levels were significantly increased in pancreatic and colorectal Ca groups ($p< 0.05$) and natural killer (CD16/CD56) lymphocytes were also significantly higher in gastric Ca, colorectal Ca and HCC patients compared to the controls ($p< 0.01$-0.05). Besides, CD19 lymphocytes were significantly lower in the pancreatic and colorectal Ca groups ($p<0.01$-0.05). CD3 and CD4 lymphocyte levels were not significantly different in all cancer groups.*

CONCLUSİON: *Cellular immunity did not weaken, even activated in the course of gastrointestinal carcinogenesis but, this activation seems insufficient in the fight against GI cancers. Also, we observed severe decline of humoral immunity parameters associated with the progress of carcinogenesis. Strengthening humoral immune system must be considered in the future strategy of the cancer immunotherapy.*

KEY WORDS*: Gastrointestinal cancers, cellular and humoral immunity.*

INTRODUCTION

Cancer is an historical and major global health problem around the world. Gastrointestinal cancers (GI Ca) are the leading cause of high human morbidity and mortality among all cancer groups. Immune system has been well organized to develop humoral and cellular immune responses against both pathogen microorganisms and tumor cells. Novadays, immunological fight against cancers constitutes an essential topic of immunology.

Cancer cells produce many mutant or aberrant proteins with specific antigenic properties that are captured, processed by dendritic cells (antigen presenting cells "APC or DC") and presented to host $CD8^+$ T lymphocytes. These cytotoxic and class I MHC- restricted lymphocytes (CTL) are stimulated and specialized to recognize peptides derived from mutant or aberrant proteins and they will kill the cancer cells expressing these proteins. In addition; $CD4^+$ helper T lymphocytes stimulated by DC, produce some cytokines (IL-2, IFN-ɣ, TNF etc.) and provide the specialisation and effectiveness of CTL for tumor lysis. Natural killer (NK) lymphocytes (CD 16/56 etc.), stimulated by antigen-activated $CD4^+$ helper T lymphocytes, differentiate into lymphokine-activated killer (LAK) cells which nonspecifically lyse tumor cells. NK lymphocytes may also kill directly cancer cells that evade CTL- mediated lysis by reducing expression of class I MHC molecules. In animal studies concerning T cell-deficiency, NK cells alone may play an important role in immunosurveillance against developing tumors (1,2).

In our study we aimed to research the parameters of the humoral and cellular immune systems in the gastric, pancreatic, colorectal and hepatocellular cancer patients.

MATERIALS AND METHODS

Analysis of Mononuclear Cell Subsets

The expressions of cellular and humoral immune parameters (CD3, CD4, CD8, CD16/CD56 and CD 19 on peripheral blood T and B lymphocytes respectively) were analyzed in the venous blood samples of GI cancer patients by flow cytometry method in the department of Gastroenterology, Faculty of Medicine, Bezmialem Vakıf University. Group I consists of 197 gastrointestinal cancer (71 gastric, 50 pancreatic, 27 colorectal and 50 HCC) patients and Group II consists of 30 healthy persons as control group.

Samples of venous peripheral blood were drawn into EDTA tubes from all subjects after obtaining informed consent. All samples were stored at 2-8 °C from the time they were taken in the test laboratory until the time of recording and staining (no more than 24 hours after the draw). To each 0.1 ml of blood sample, 1 ml of 25 °C degree BD Facs Lysing solution was added to break down the red blood cells. It was left incubated at room temperature for 4 minutes on a roller and centrifuged at 1600 RPM for 4 minutes. The supernatant carefully aspirated then resuspended pellet in approximately 2 ml 1xPBS at room temperature. Then it was again centrifuged for 4 minutes at 1600 RPM and then the supernatant was aspirated. For cell surface fluorescence staining, aliquots of 50 μL of cells of peripheral blood mononuclear cell suspension were incubated for 40 min at room temperature with the appropriate monoclonal antibodies. The data were obtained using a BD FACS Canto II flow cytometer. The flow cytometry was established using BD Cytometer Setup and Tracking (CS&T) beads. BD FACS Diva™ software (v8.0.1) was used for count and analysis. Data analysis was done using autoanalysis in BD FACS Diva software and

graphic layers for surface labels and control antibodies were created using an FCS Express templet. After the total analysis, the data was automatically transferred to Excel 2013 in column and row shape.

Statistical method: One way Anova and post hoc Dunnett tests were used for comparison of mean lymphocyte values (%) of cancer groups with the healthy control group. Significance level is determined as $p< 0.05$.

RESULTS:

Serum CD3, CD4, CD8, natural killer (CD16 / CD56) and CD 19 lymphocyte values were presented in Table 1.

Table 1. Cellular and humoral immune parameters (IMP) in GI and HCC patients and controls

IMP (%)	G. Ca (Mean ± SD)	P. Ca (Mean ± SD)	CR. Ca (Mean ± SD)	HCC (Mean ± SD)	Control (Mean ± SD)
CD3+ lymph.	68.03 ±13.89	74.06 ± 12.35	75.09 ± 9.74	69.94±11.10	71.65 ± 8.59
CD4+ lymph.	40.71 ± 10.67	30.44 ± 13.33	43.72 ± 14.02	38.20± 13.53	43.03 ± 8.88
CD8+ lymph.	26.79 ± 10.94	[a] 26.91 ± 14.11	[a] 31.33 ± 11.81	25.83 ± 12.00	23.50 ± 7.88
CD19+ lymph.	10.18 ± 7.35	[a] 8.37 ± 4.44	[b] 6.78 ± 3.51	12.64 ± 10.15	12.82±5.89
NK (CD16/56)lymph.	[b] 14.67 ±10.33	13.30 ± 7.23	[b] 17.92 ± 9.73	[a] 14.72±9.49	8.39 ± 5.46

Abbreviations: a: $p<0.05$, b: $p<0.01$. IMP: Immune Parameters, G.Ca: Gastric cancer, P.Ca: Pancreatic cancer, CR. Ca: Colorectal cancer, HCC: Hepatocellular cancer, NK: Natural killer lymph.: lymphocytes, Data are presented as mean ± SD.

CD3 and CD4 lymphocyte levels were similar in all cancer groups compared to the control group ($p> 0.05$). CD8 lymphocyte levels were significantly increased in pancreatic and colorectal Ca groups ($p< 0.05$) and they were also elevated in gastric and HCC patients numerically (Figure 1).

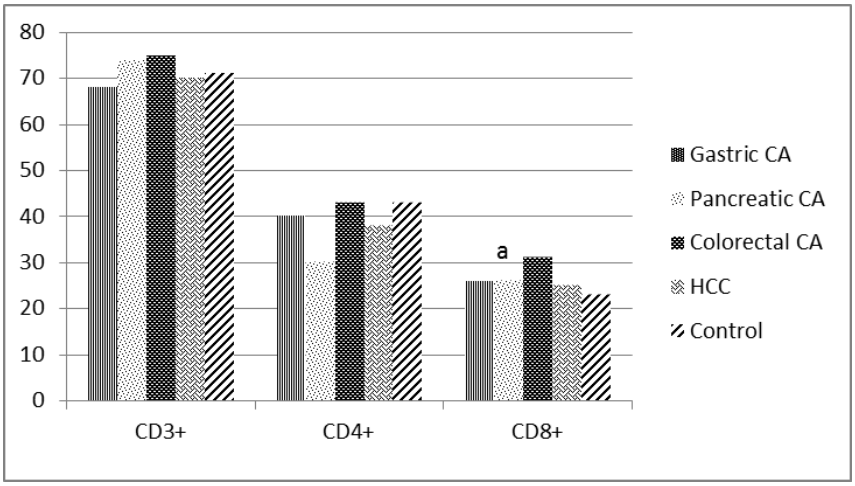

Figure 1. Serum CD3, CD4 and CD8 lymphocytes levels (%) in GI Ca and HCC patients

Natural killer (CD16 / CD56) lymphocytes were significantly higher in gastric Ca, colorectal Ca and HCC patients compared to the controls ($p< 0.01$) and they were also elevated in pancreatic Ca numerically (Figure 2).

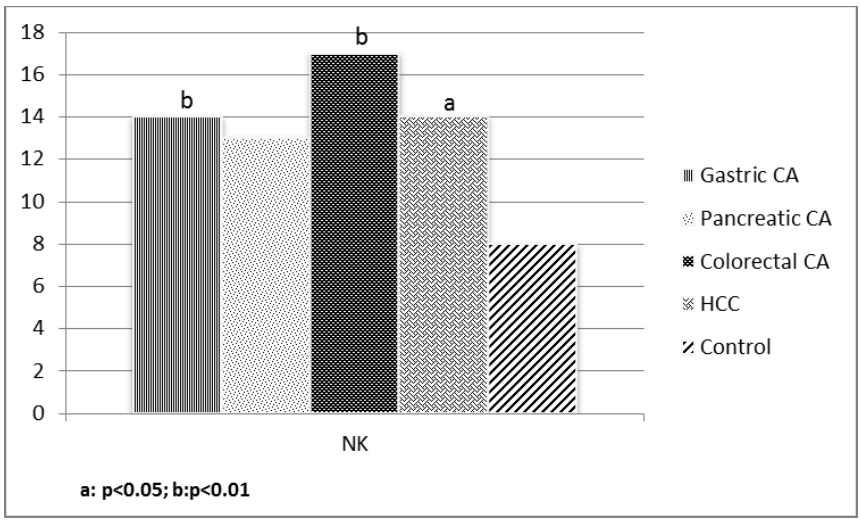

Figure 2. Serum NK lymphocytes levels (%) in GI Ca and HCC patients

CD19 lymphocytes were significantly lower in colorectal and pancreatic Ca groups ($p< 0.01$). Also, they were lower numerically in gastric Ca group than in the control group (Figure 3).

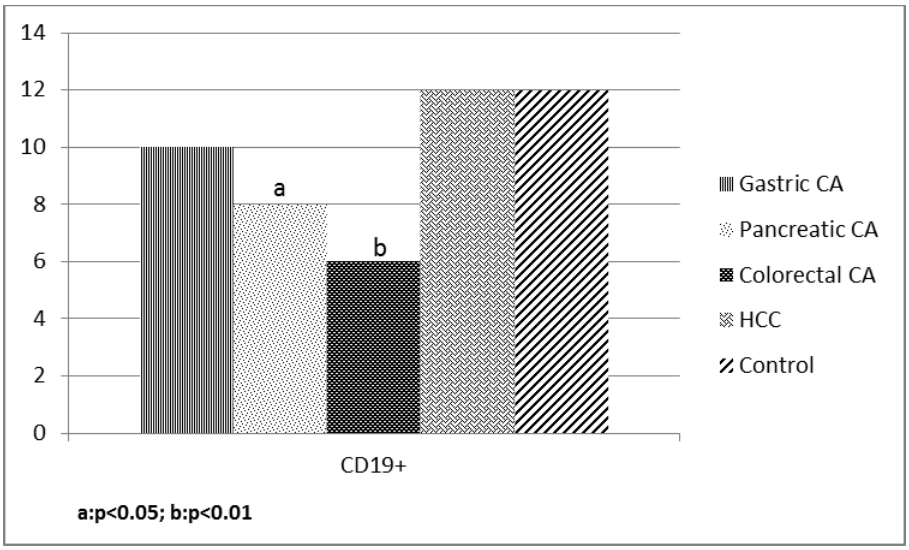

Figure 3. Serum CD19 lymphocytes levels (%) in GI Ca and HCC patients

DISCUSSION

Novadays, Immunotherapy is becoming increasingly important and successful in cancer treatment.

Cancer immunotherapy consists of the use of immunomodulatory monoclonal antibodies (Anti-CD 20 etc.) or checkpoint inhibitors (Anti-PD-1 / PD-L1 "nivolumab, pembrolizumab", anti-CTLA-4 "ipilimumab"), adoptive cell transfers (cytokine induced cytotoxic T lymphocytes etc.) and dendritic cell-based anticancer vaccines (3). Scientific studies revealed the existence of naturally occurring cytolytic CD8 tumor infiltration lymphocytes (TIL) specifically reactive to metastatic gastrointestinal cancers. Higher numbers of T-bet+ intratumoral lymphoid cells (CD4, CD8 and CD56) were correlated with good survival and lower numbers of T-bet+ TILs were associated with poor prognostic findings in gastric cancer (4,5).

Also, the significantly low numbers of intrahepatic CD56 lymphocytes were observed in gastric and colorectal cancer patients with liver metastases, which reflects the essential role of NK cells in the hepatic immune control of metastatic malignancies (6).

In a study on blood lymphocyte populations in gastrointestinal cancer patients, CD4 cells decreased both in gastric and colorectal cancers as the stages progressed, whereas CD8 levels tended to increase in advanced stages (7).

In patients with pancreatic cancer, the presence of both CD8 and CD4 T cells in the tumor is related with good prognosis and survival. However, when pancreatic premalign lesions progress to malignant phase, CD8 effector cells decreased in number while the immunosuppressive Treg (CD4+CD25+FoxP3+) cells increased in the tumor microenvironment (8-10).

In a metastatic pancreatic cancer which regressed following treatment with the immune modulator anti-CTLA-4 (Ipilimumab), natural killer (NK) cells were predominated among the TIL (92% CD56+) in extracted cancer deposit. These NK cells were capable of lysing many types of malign cells (11).

In the studies on colorectal cancer (CRC) without metastasis, intratumoral T CD8+ and CD45RO+ memory lymphocytes' infiltrate has shown to be correlated with increased survival. The proportion of activated TILs decreased significantly along tumour stage (from stage II through stage III-IV), showing functional decrease in immunosurveillance associated with CRC progression (12,13). Also, an extensive infiltration of NK cells has been reported to be protective against colon cancer-initiating cells and associated with a good prognosis (14).

In HCC patients, the high numbers of TILs were correlated with increased survival after surgical resection of tumors. Also, the high CD4+/CD8+ T cell ratio was related with a decreased risk of tumor recurrence after liver transplantation for HCC. NK cells also constitute an essential defense component of the liver microenvironment. The numbers of both peripheral blood and liver CD56 NK cells were also diminished in HCC patients (15,16).

The active function of the humoral immunity is also essential like cellular immunity in the fight against the cancer. Interestingly, the acquise of the humoral immune activity to viral infections in childhood may be protective against cancer development (17). Experimental animal studies support the hypothesis that cancer protection induced by

infection is mediated by immune responses against multiple self antigens which were homologues of previously identified human tumor antigens (18).

Many monoclonal antibodies have been discovered in cancer patients by using human hybridoma technology. Nearly all tumor-specific antibodies are pentameric IgMs related to the innate immunity and are synthesized by CD5+ B-cells. Some human monoclonal antibodies (SAM-6, SC-1, PAM-1 etc.) have been isolated from gastric cancer patients and their use in experimental studies and in patients revealed a decrease in both tumor weight and volume associated with high apoptotic activity (19,20). Also, some monoclonal antibodies (anti-CTLA-4 "ipilimumab or tremelimumab", anti-PD-L1, anti-PD-1) developed against molecules inhibiting cytotoxic T lymphocytes increases T-cell activation and proliferation in vivo by checkpoint blockade and have been effective in combination with other treatment modalities in advanced human malignancies (1,2,21).

In our study, we detected significant increase of cellular immune parameters in gastrointestinal cancers. CD8 T lymphocytes increased in pancreatic and colorectal cancer patients and NK (CD16/CD56) lymphocytes increased in gastric, colorectal and hepatocellular cancer patients significantly. These findings show that cellular immunity did not decline but, on the contrary, stimulated during the course of the GI and hepatocellular cancers. However, the advanced stage of the carcinogenesis suggests that tumor antigens have not been recognized and tumor cells have not been killed sufficiently by the cellular immune system despite its activation ("tumor evasion and escape"). Interestingly, we observed the significant decrease of the CD19 lymphocytes (humoral immune parameters) both in pancreatic and colorectal cancer groups. Also, CD19 lymphocytes were lower numerically in the gastric cancer group than in the control group. The decrease of the humoral immune activity in inoperable GI cancers reveals that the carcinogenesis progress associated with severe decline in humoral immunity. This fact implies the importance of the reanimation of the humoral immune system for a successful fight against GI cancers.

In conclusion**,** cellular immunity did not weaken, even activated in the course of gastrointestinal carcinogenesis possibly related to malign stimulation. But this activation is not sufficient for the prevention of the GI carcinogenesis progression because of tumor evasion or escape mechanisms. On the other hand, we observed severe decline of humoral immunity in advanced gastrointestinal cancers associated with the progress of carcinogenesis. Strengthening humoral immune system by various humoral immune methods (specific monoclonal and antitumoral antibodies, etc…) must be considered principally in the therapeutic strategy of advanced gastrointestinal cancers.

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Conflicts of Interest:

All authors have no conflict of interest to report.

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