Investigation of IL6, IL10, IFN-γ and TNF-α at Iraqi patients with colon cancer suffering type-2 diabetes

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ABSTRACT

Objective: Diabetes mellitus is one of the risk factor for all cause of cancer such as colorectal cancer most common among females worldwide. Aimed to investigate the serum levels of IL-6, IL-10 andIFN-γ, TNF-α in patients with colon cancer suffering diabetes mellitus.

Materials and methods: Fifty patients with colon cancer (21 female and 29 male) with ages ranged between (20-70) years were taken from (Al-Hussain Hospital City/Kerbala, Digestive and Liver Disease /Education Hospital Medical City Baghdad and Teaching Oncology Hospital /Baghdad Medical City/ Baghdad /Iraq). Compared with 20 healthy persons. Estimation of CEA, Ca19-9 by Vidas, soTNF-α, IFN-γ, IL-6 and IL-10 were studied using the enzyme-linked immunosorbent assay (ELISA) method.

Results: Thirty patients among fifty patients of colon cancer (60%) of patients showed a high diabetes mellitus, fasting plasma glucose was ≥ 126 mg/dl.Twenty non-diabetic patients of colon cancer were (40%),observed a significant (p<0.05) increase when comparing the levels of IL-6 and TNF-α in diabetic age group (40-59) yrs with age group (60-70)yrs. In the present study, the results revealed a significant (p<0.05) decrease in the level of IFN-γ when compared no diabetic age group (40-59) with diabetic age group (60-70) yrs.

Conclusion: Elevated (CEA ,Ca 19-9 , IL-6, IL-10, IFN-γ and TNF-α)levels were observed in patients with colon cancer that suffering type 2 diabetes , those patients have high risk of developing diabetes ,these tumor markers and cytokines have a role in diagnosisinterpretation this puzzle in patients.

Keywords: IL-6, IL-10, IFN-γ, TNF-α, Colon cancer, Diabetes.

INTRODUCTION

Type 2 diabetes mellitus is a metabolic disease in which genetic and environmental factors interact in determining impaired β-cell insulin secretion and peripheral insulin resistance [1].

Diabetes mellitus is one of the risk factor for all cause of cancer such as colorectal cancer and it have a worse disease-free survival than those who do not have diabetes mellitus, but the impact of diabetes mellitus on colorectal cancer prognosis is not clear [2].

Carcinoembryonic antigen (CEA) and carbohydrate antigen Ca 19-9 are most often known as the common tumor markers of colon cancer, and it's levels are used not only for the preoperative assessment of extent and outcome of cancer, but also for postoperative monitoring of recurrence. Combined data on preoperative increases in CEA and Ca 19-9 in sera can help predict the prognosis of patients with colorectal carcinoma [3].

Both CEA and Ca 19-9 are considered to indicate an advanced stage in colorectal cancer patients [4].

Cytokine regulation of human colorectal cancer is not clearly understood. However, it has been postulated that proinflammatory cytokines strongly influence the immunological status of colorectal cancer [5].

Cytokines are released responded to a diverse range of cellular stresses such as carcinogen-induced injury, infection and inflammation [6].

IL-6 is an important tumor promoting cytokine that imposesproliferation and anti-apoptotic effects in tumor cells [7].
IL-10 is an immunoregulatory cytokine and the biological function of IL-10 is limitation and termination of inflammatory responses, it isregulated the differentiation and proliferation of several immunecells [8].

The increased levels of TNF-α have a role in the development of cancer in many cancer types [9].

IFN-γ stimulates many anti-proliferative and tumoricidal biochemical pathways in macrophages and other cells. This an evidence increasingly indicates that it is also involved in the development of tumor immune escape. Production of IFN- γ released within the forward-regulatory cascade of Th1-type immune response in order to restrict the growth of malignant cells is associated with the development of immunodeficiency and a chronic inflammatory state persists [10].

In this study was aimed to estimate the level IL-6, IL-10, IFN-γ and TNF-α at Iraqi patients with colon cancer suffering type-2 diabetes.

MATERIALS AND METHODS

Patients and Methods:
2.1. Selection of patients:
During the period 1/October/2013 to 1/March/2014, fifty patients with colon cancer (21 female and 29 male) and thirty patients have diabetes mellitus type 2 with ages ranged (20-70) years were taken from (Al-Hussain Hospital City/Kerbala, Digestive and Liver Disease /Education Hospital Medical City Baghdad and Teaching Oncology Hospital /Baghdad Medical City/ Baghdad /Iraq).

Control group consisted of 20 healthy people who were free from signs and symptoms of cancer that matched in age and gender with patients, and had no history for any gastrointestinal problem.

2.2. Sample collection and assay procedure:
Blood sample (5ml) was collected left at room temperature and then centrifuge for 15 min. at (3000 rpm). Serum was then separated and freezes until time of analysis. Estimation of CEA,Ca19-9 Vidas(Biomerieux SA/France),IL10, IL6, IFN-γ and TNFα ELISA kit (Cusabio/China) in serum using commercially available and performed as recommended in leaflet with kit,and examinedfasting glucose levels.

2.3. Statistical analysis:
Results are expressed as mean ± standard deviation (SD) and standard error (SE), student t-test and ANOVA used to analyze

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results by using SPSS version 22. P-value ≤ 0.05 was considered significant.

RESULTS

A total of 50 patients with colon cancer divided into three groups according to the age (20-39yrs) 13(18.6%), (40-59yrs) 26(37.1%) and (60-70yrs) 11(15.7%). Thirty patients among fifty patients of colon cancer (60%) of patients showed a high diabetes mellitus, fasting plasma glucose was ≥ 126 mg/dl.Twenty non-diabetic patients of colon cancer were (40%). The clinical characteristics of patients with colon cancer were shown in **Table 1**, **Table 2** was indicated that CEA levels was increase significantly (p<0.001) in diabeticpatients which compared with control group and significant increase (p<0.001) when compared patients between diabetic and non-diabetic patients, whereas there was a significant (p<0.001) increase in Ca 19-9 at patients with diabetic patients compared with control group and a significant (p<0.05) increase between diabetic patients and non diabetic patients. Also, the results revealed a significant (p<0.001) increase in the levels of IL-10, IL-6, IFN-γ and TNF-α in diabetic patients compared with control group. So, the results indicated a significant (p<0.001) increase in IL-10 when compared between diabetic patients and non diabetic patients. In other side, IL-6, IFN-γ and TNF-α showed significantly (p<0.05) increase at diabetic patients compared with non diabetic patients.

Patients were analyzed according to the age groups into diabetic and non diabetic patients, the percentage were 26% of non diabetic in age group (20-39yrs), diabetic38% and 14% non diabetic patients were in age group (40-59yrs), whereas 22% diabetic patients in age group (60-70yrs). The levels of CEA, Ca19-9, IL-10, IL-6, IFN-γ and TNF-α showed no significant (p>0.05) difference in age group (20-39yrs) that compared with age group (60-70yrs), So, the results revealed no significant difference (p>0.05) when compared diabetic with non diabetic patients in age group (40-59yrs) excepted for IFN-γ that showed a significant (p<0.001) increase in the same age group. Also, there was no any significant (p>0.05) difference in all measured parameters when compared non diabetic and diabetic patients in age group (20-39yrs), (40-59yrs) respectively. There was no any significant difference when compared the all measured parameters in age group (20-39yrs) with no diabetic patients age group (40-59yrs) excepted for IFN-γ that showed a significant (p<0.05) increase in the same compared group. Therefore, we observed a significant (p<0.05) increase when comparing the levels of IL-6 and TNF-α in diabetic age group (40-59yrs) with age group (60-70yrs) and no significant (p>0.05) variance in the others parameters. In the present study, the results revealed a significant (p<0.05) decrease in the level of IFN-γ when compared no diabetic age group (40-59yrs) with diabetic age group (60-70yrs) and no significant (p>0.05) variance to the others parameters (**Table 3**).

Table No. 1: The clinical characteristics of patients with colon cancer

Variable	No.	Percentage (%)
Total number of patients	50	100
Age		
20-39 yrs	13	18.6
40-59 yrs	26	37.1
60-70 yrs	11	15.7
Gender		
Female	21	42
Male	29	58
Diabetes patients	30	60
Non diabetes patients	20	40

Table No. 2: The levels of parameters in control and patients

Group	No.	CEA Mean ± SD	Ca 19- 9 Mean ± SD	IL-10 Mean ± SD	IL-6 Mean ± SD	IFN-γ Mean ± SD	TNF-α Mean ± SD
Control	20	0.8 ± 0.01[1]	5 ± 0.4[1]	9.8 ± 7.7[1]	5 ± 2.5[1]	3 ± 2[1]	87 ± 36[1]
Patients:							
Diabetes	30	12 ± 6	31.5 ± 4	59 ± 50	19 ± 7	52.3 ± 7.7	130.5 ± 37
Non diabetes	20	2 ± 0.5[2]	10 ± 6[3]	16 ± 9[2]	14 ± 6[3]	5 ± 0.9[3]	97 ± 57[3]

[1]Significant control vs. patients with diabetes (p<0.001); [2]Significant Diabetes patients vs. non diabetes patients (p<0.001); [3]Significant Diabetes patients vs. non diabetes patients (p<0.05)

Table No. 3: The levels of parameters in patients according to the age group

Age groups	State	No.(%)	CEA(IU/ml) Mean±SE	Ca19-9(U/ml) Mean±SE	IL10(pg/ml) Mean±SE	IL6(pg/ml) Mean±SE	IFN-γ Mean±SE	TNF-α (pg/ml) Mean±SE
Age (20- 39)yrs	No diabetes	13(26%)	5±1.6[1]	10±5[1]	29±9[1]	16.5±2[1]	20±7[1]	135.5±8[1]
Age (40-59)yrs	Diabetes	19(38%)	9±3[3]	26±10[3]	42±10[3]	15±2[3]	24.6±7[3]	140±7[3]
	No diabetes	7(14%)	6±3	25±21	68.8±30	17.5±5	130±57[2,4]	116±17
Age (60-70)	Diabetes	11(22%)	13±5	33±11.5	35.7±11.5	21±1.4[5]	13±4[6]	113±12[5]

[1]no significant (p>0.05) difference in age(20-39)yrs vs. age(60-70) yrs, [2]diabetes age (40-59) yrs vs. no diabetes age (40-59) yrssignificant at (p<0.001), [3]no significant (p>0.05) difference in age(20-39)yrs vs. diabetes age(40-59) yrs, [4]age(20-39)yrs vs. no diabetes age(40-59) yrssignificant at (p<0.05), [5]diabetes age (40-59) vs. diabetes age (60-70) yrs significant at (p<0.05), [6]no diabetes age(40-59) vs. diabetes age (60-70) yrs significant at (p<0.05)

DISCUSSION

Our study was reckon that type 2 diabetic was a portent for colorectal cancer, high concentrations of glucose conduced to elevated in CEA, Ca19-9, IL-10, IL-6, IFN-γ and TNF-α,but this study be left most of which remain arguable.

Researchers believed that diabetes-related factor associated with increased risk of breast and colon.

Cancer Insulin use was associated with increased colon cancer incidence in women, but not with colon cancer in men or breast or prostate cancer risk [11].

Other study referred significantly relevance of CEA, Ca19-9, IL-10 and TNF- α with colon cancer [12].

This study agreement with other study was demonstrated that colon cancer effects may be mediated mostly by insulin [13].

Diabetes is associated with higher risk of death from all causes and cancer [14]. In this study, incidence of colorectal cancer

related with diabetes in elderly patients (40-70yrs). Diabetes increased risk among geriatric patients with colorectal cancer, especially among cancer patients who had diabetes with complications. The increased risk by increased cardiovascular-specific mortality, not by increased colorectal cancer-specific mortality [15].

Others suggested that hyperglycemia down-regulates CD33 expression and triggers thespontaneous secretion of TNF-a by peripheral monocytes, that is importance of blood glucose control for maintaining innate immune function and suggest the participation of CD33 in the inflammatory profile associated with type 2 diabetes [16], so perceived over production of proinflammatory (IFN-γ) and inflammatory (TNF- α) cytokines in addition to low level of IL-10 could play a crucial role in pathogenesis of HCV that leads to Type2 Diabetes [17].

The increased concentration of IL-10 in the serum samples from Diabetes Mellitus patients with Diabetic Nephropathic seems to depend on the severity of the nephropathy. The excessive IL-10 production may indirectly contribute towards progression. On the other hand, it may explain the relatively long course of diabetic nephropathy [18].

Researchers were believed that insulinopenic may be the reason for immunological defects [19]. Other studies suggested diabetes is a significant effect factor for cancer mortality, independent of obesity [14].

Functions of IL-10 regulatory responses are related to awkward clearanceof pathogens and tumor cells, eventuated in chronic infections and tumor development [20].

High glucose triggers several direct and indirect mechanisms that cooperate topromote cancer cell proliferation, migration, invasion and immunological escape [21].

This study lead to elucidationof new intense rapport for diabetes-associated cancers, presumed hyperglycaemialed to insulin secretions that induced cytokines production through inflammation subsequently tumor proliferations.

CONCLUSION

Elevated (CEA ,Ca 19-9 ,IL-10, IL-6, IFN-γ and TNF-α) levels were observed in patients with colon cancer that suffering type 2 diabetes, those patients have high risk of developing diabetes ,these tumor markers and cytokines have a role in diagnosisinterpretation this puzzle in patients , more studies are needed to explication genetic correlation between type 2 diabetes and colon cancer.

ACKNOWLEDGMENT

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