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Studying of Some Immunological Parameters in Gastric Cancer Patients in Hilla City

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Abstract: Gastric cancer remains one of the deadly diseases with poor prognosis. White blood cells count and immunological cytokines are highly expressed in the gastric mucosa and potently activates Th2 immunity. In Hilla city there is no previous study dealing with the association between some immunological parameters and stages of gastric carcinoma. The present study aims to investigate the relationship between some immunological parameters in both six of patients with different age groups with the stages of gastric cancer.

Methods: This study involved seventy subjects, forty six of them were suffering from gastric carcinoma with (69.6%) of *H. pylori* positive infection (+ve) which divided into two groups male (n=23) and female (n=23) each group subdivided into six groups according to the age [(≥ 20), (21-30), (31-40), (41-50), (51-60), (61 \leq)] male and female in sequence and into four groups according to the stages of disease (stage I, stage II, stage III and stage IV) male and female in sequence. Healthy control group include twenty four subjects which divided into two groups male (n=12) and female (n=12) were not receiving any medications and not have any history of chronic or acute illnesses.

Blood specimens were collected for studying the white blood cells count and their differential types (lymphocytes, monocytes, basophils, eosinophils, neutrophils and white cells) by using CELL—DYN Ruby hematology analyzer, also we studying the levels of IL-10 and IL-33 by automated microtiter plate ELISA reader.

Results : The results explained a significant increased (p<0.05) of the gastric cancer in male than female in two age groups [(41-50), (51-60)] as follow [n=7, (15.21%) & n=6, (13.04%)] respectively in male and [n=6, (13.04%) & n=5, (10.86%)] respectively in female, also we measured the white blood cells count and we found a significant increased (p<0.05) in the number of all differential cell types in male than female groups compared with an increased in their numbers in all stages of disease in both sex of gastric carcinoma. We saw a significant increased in the concentrations of IL- 10 and IL- 33 in patients of both sex compared with healthy control groups with progression of their concentrations respectively in four stages of disease.

Conclusions : We can conclude that the significant increased in the white blood cells count occur during several cases linking to inflammation and progression of cancer. IL- 10 and IL- 33 are regarded as an impotent and a prognostic immunological parameters for gastric carcinoma. **Keywords :** Gastric carcinoma; white blood cells counts; IL- 10; IL- 33.

Introduction

Gastric cancer, also called stomach cancer, is a cancer that starts in the stomach. It is tend to develop slowly over many years by pre-cancerous changes often occur in the inner lining (mucosa) of the stomach. Cancers starting in different sections of the stomach may cause different symptoms and tend to have different outcomes. The cancer's location can also affect the treatment options^[1&28]

A number of factors probably influence an individual's predisposition to gastric cancer and course of progression to it like gender, age, geography, *Helicobacter pylori* infection, diet, smoking, being overweight or obese. stomach surgery, pernicious anemia, genetic and familial risk factors etc....^[2&26]

There are different types of *gastric* cancer include:

Adenocarcinoma: These cancers develop from the cells that form the innermost lining of the stomach Lymphoma: These are cancers of the immune system tissue that are sometimes found in the wall of the stomach. Gastrointestinal stromal tumor (GIST): These are rare tumors that start in very early forms of cells in the wall of the stomach called *interstitial cells of Cajal*. Some of these tumors are non-cancerous (benign); others are cancerous. Carcinoid tumor: These are tumors that start in hormone-making cells of the stomach. Most of these tumors do not spread to other organs. Other types of cancer, such as squamous cell carcinoma, small cell carcinoma, and leiomyosarcoma, can also start in the stomach. ^[3&29]

The clinical stages of stomach cancer are: **Stage 0.** Limited to the inner lining of the stomach. **Stage I.** Penetration to the second or third layers of the stomach. **Stage II.** Penetration to the second layer and more distant lymph nodes, or the third layer and only nearby lymph nodes, or all four layers but not the lymph nodes. **Stage III.** Penetration to the third layer and more distant lymph nodes, or penetration to the fourth layer and either nearby tissues or nearby or more distant lymph nodes. **Stage IV.** Cancer has spread to nearby tissues and more distant lymph nodes, or has metastasized to other organs.^[4]

Recent researches have provided that *H. pylori* bacteria, particularly certain subtypes, can convert substances in some foods into chemicals that cause mutations (changes) in the DNA of the cells in the stomach lining^[30]. This may also explain why certain foods such as preserved meats increase a person's risk for stomach cancer. On the other hand, some of the foods that might lower stomach cancer risk, such as fruits and vegetables, contain antioxidants that can block substances that damage a cell's DNA. People with stomach cancer have a higher rate of *H. pylori* infection than people without this cancer. *H. pylori* infection is also linked to some types of lymphoma of the stomach and the infection progresses over decades through stages of chronic gastritis, atrophy, intestinal metaplasia, dysplasia, and cancer. ^[5&6]

The systemic and local cytokine environment may modulate the immunogenicity and affect anti-tumor immune function of tumor-infiltrating lymphocytes. Focusing on individual cytokines has generated evidence that pro-inflammatory cytokine and anti-inflammatory cytokines may have a complex role in gastrointestinal carcinogenesis.^[7]

The roles of different cytokines in regulating antimicrobial immunity and inflammation make them attractive candidates for being genetic host markers in evaluating individual susceptibility to gastric cancer development^[8]. IL-10 is an immunoregulatory cytokine and its main biological function is limitation and termination of inflammatory responses. IL-10 also regulates differentiation and proliferation of several immune cells^[9]. Thus, its dual role as immunosuppressive and antiangiogenic cytokine may have both promoting and inhibiting effect on tumors development and progression. ^[10&27]

IL-33 is highly expressed in the gastric epithelium and therefore may be an important factor in limiting *H. pylori* colonization and consequent inflammatory pathology. It is a member of the IL-1 family and activates multiple immune regulatory cells including group 2 innate lymphoid cells (ILC2), basophils, mast cells, eosinophils, natural killer T cells, and Th2 lymphocytes. and it play an important role in tumorigenes by promoting endothelial permeability and angiogenesis^[11].

Aim: The aim of this study is to investigate the relationship between some immunological parameters (White blood cells count with differential types and immunological cytokines IL-10 & IL-33) in both six of patients with different age groups and in different stages of disease compared with healthy controls.

Materials and Methods

1- Patient groups

Forty six patients suffering from gastric cancer were admitted to the Oncology unit of Medical Marjan City in Babylon province during the period from January till March 2016. All patients were diagnosed by specialist physician which divided into two groups male (n=23) and female (n=23) each group subdivided into six groups according to the age [(≥ 20), (21-30), (31-40), (41-50), (51-60), (61 \leq)] male and female in sequence and into four groups according to the stages of disease (stage I, stage II, stage III and stage IV) male and female in sequence.

2- Healthy control group

Healthy control group include twenty four subjects which divided into two groups male (n=12) and female (n=12) were not receiving any medications and not have any history of chronic or acute illnesses.

3- Specimens

Blood specimens were collected with anticoagulant from patients and healthy control groups for studying the white blood cells count and the serum levels of IL- 10 and IL- 33.

4- White blood cells count

The white blood cells count and their differential types (lymphocytes , monocytes , basophils , eosinophils , neutrophils and white cells) were detected by using CELL—DYN Ruby hematology analyzer (Analyzer S/N: 35901BG, U.S.A).

5- Immunology

We measured the serum levels of cytokines (IL- 10 and IL- 33) by automated microtiter plate ELISA reader. The IL- 10 and IL- 33 cytokines kits were provided from (Ray Bio, USA, Company).

Results

The results explained that (69.6%) of gastric cancer patients were *H. pylori* positive infection (+ve) as in table (1).

Table (1) *H. pylori* infection in gastric cancer patients

H. pylori infection	No. Of patients	%
<i>H. pylori</i> +ve	32	69.6
H. pylori -ve	14	30.4
Total	46	100

The high rate of risk factors in gastric cancer patients were represented in positive family history about (39.13%) followed by smoking about (28.26%) table (2),

Table (2) Risk factors for gastric cancer patients

Risk factor	No. of patients	%
Smoking	13	28.26
Alcohol	9	19.57
conception		
Food intake types	6	13.04
Positive family	18	39.13
history		
Total	46	100

Also we saw an increased in the gastric cancer in male than female in two age groups [(41-50), (51-60)] as in table (3)

Age group	Male	%	Female	%
(years)				
≥ 20	1	2.17	1	2.17
21-30	3	6.52	3	6.52
31-40	3	6.52	4	8.69
41-50	7	15.21	6	13.04
51-60	6	13.04	5	10.86
<u>61</u> ≤	3	6.52	4	8.69
Total	23	49.98	23	49.97

Table (3) Gastric cancer patients according to the age in male and female groups

In addition to see a significant increased (p<0.05) in the number of white blood cells count with their differential types in gastric cancer patients in male rather than female compared with control groups as in table (4).

Table (4) White blood cells count with their differential types in gastric cancer patients

WBC count	Male	P- value	Female	P- value	Control	P- value
10e3/uL					group	
M±SD						
White cells	18.373±2.106	0.020^{a}	16.284±0.958	0.000^{b}	6.982±1.072	0.000°
Neutophils	12.345±0.656	0.030 ^a	11.255±1.243	0.100 ^b	5.011±0.341	0.000°
Eosinophils	0.361±0.182	0.020^{a}	0.283±0.032	0.000^{a}	0.243 ± 0.082	0.000^{a}
Basophils	0.531±0.161	0.010^{a}	0.145±0.043	0.000^{b}	0.098 ± 0.082	0.000°
Monocytes	2.024 ± 1.011	0.020^{a}	2.016 ± 2.457	0.020^{b}	0.720±1.221	0.000°
Lymphocytes	3.381±1.316	0.030 ^a	2.218±1.218	0.100^{a}	1.985 ± 0.432	0.000^{b}

Table (5) show a significant increased (p<0.05) in the number of white blood cells count with all differential cell types in male than female groups in all stages of gastric carcinoma while we did not have any result of patients at 0 stage of disease because we did not obtain patients at this stage.

Table (5) White blood cells count with their differential types in gastric cancer patients according to the stages of disease

WBC count	Stages of gastric cancer disease							
10e3/uL	Ι	р-	II	р-	III	р-	IV	
M±SD		value		value		value		
White cells	15.279±1.001	0.050^{a}	15.893±0.062	0.000^{b}	18.221±1.003	0.030 ^c	20.978±10.23	
							0	
Neutophils	7.025±0.129	0.120^{a}	7.198±1.340	0.040^{b}	9.223±0.653	0.000°	11.834±0.232	
Eosinophils	0.262±0.134	0.030^{a}	0.312±0.121	0.042^{b}	0.632 ± 0.060	0.010°	0.965 ± 0.432	
Basophils	0.192±0.296	0.120^{a}	0.232±0.175	0.050^{b}	0.754±0.229	0.050°	0.951±0.320	
Monocytes	0.961±0.023	0.010^{a}	1.973±0.190	0.020^{b}	3.524±0.613	0.050°	5.912±1.321	
Lymphocytes	2.635±1.983	0.040^{a}	2.954 ± 1.498	0.010^{b}	3.428±1.948	0.050°	4.545±2.324	

Also we found a significant increased (p<0.05) in the concentrations of IL- 10 and IL- 33 in patients of both sex compared with healthy control groups as in table (6)

Gender	Cytokine concentrations (pg/ml)							
		IL- 10		IL- 33				
	Patients group	P-value	Control groups	Patients group	P-value	Control groups		
Male	337.943±11.432	0.000^{a}	49.943±9.873	695.502±8.321	0.000^{a}	50.412±13.210		
Female	199.089±10.432	0.010 ^a	35.787±3.452	493.579±4.579	0.100 ^a	42.311±12.895		

Table (6) Concentrations of IL- 10 and IL- 33 in gastric carcinoma patients according to gender

Table (7) and table (8) explained a significant increased (p<0.05) in the concentrations of IL- 10 and IL-33 in male than female groups in all stages of disease.

Table (7) Concentrations of IL-10 in gastric carcinoma patients according to the stages of disease

IL-10	Stages of gastric cancer disease								
concentrati	Ι	I p- value II p- value III p- value IV							
ons (pg/ml)									
M±SD									
Male	152.871±8.566	0.310 ^a	203.432±9.071	0.140^{b}	298.361±10.643	0.230 ^c	323.432 ± 10.514		
Female	78.531±8.482	0.200 ^a	99.532±8.987	0.410 ^b	153.942±9.578	0.150 ^c	201.572±10.754		

Table (8) Concentrations of IL- 33 in gastric carcinoma patients according to the stages of disease

II-33	Stages of gastric cancer disease							
concentra	Ι	I p- value II p- value III p- value IV						
tions								
(pg/ml)								
M±SD								
Male	299.382±8.487	0.050^{a}	389.823±9.368	0.000^{b}	587.731±9.963	0.030°	629.501±10.253	
Female	226.895±8.210	0.120 ^a	259.284±8.621	0.040^{b}	432.832±8.993	0.000^{b}	495.325±9.721	

Discussion

H. pylori positively (69.6%) were detected in patients with gastric carcinoma because this bacteria increasing glandular atrophy which a precursor of gastric adenocarcinoma, and may be colonize gastric epithelium; thus, spread to the area of intestinal metaplasia with significantly associated with bile reflux and is a feature of autoimmune gastritis ^[12]. The risk of development of atrophy and cancer in the presence of *H. pylori* is again related to host and bacterial factors which influence the severity of the chronic inflammatory response. The risk is increased in subjects colonized with *cagA*-positive strains^[13&14].

Also we found that the male patients had higher prevalence compared with female and this may be associated with several factors such as age, low socioeconomic status, nutritional deficiency and poor hygiene^[15]. The highest detection rate of gastric carcinoma were (15.21%) and (13.04%) which recorded at two age groups (41-50) and (51-60) years respectively and this agreement with ^[15&16]. The excess of gastric cancer disease in positive family history for *H. pylori* infection (39.13%) and in smoker (28.26%) were agreement with ^[17,18&19] and this may be associated with aging population, changes of lifestyle, physical activates and types of diets.

The results indicated an increased in the white blood cells count and this were agreement with many researcher like ^[20&21] they found an increased in the number of these cells during inflammation and progression of cancer, and they found that these cells increased with advance stages of cancer especially white blood cells, neutrophils, monocytes and lymhocytes and this is important to study drug effect on bone marrow cell forming if the patients are treated with chemotherapy.

The increased in the concentrations of IL-10 in male with different stages of disease may be associate with several factors like: IL-10 secretion is one of the mechanisms with which the tumor cells "avoid" the

immunological surveillance, also IL-10 serum level in colorectal cancers is may be due to the functional antagonism of IL-10 toward IL-12p70 which will cause more IL-10 secretion and may be the secretion of this cytokine by the tumor cell itself to modulate the Immune system toward Th2 rather than Th1, while in gastric cancers the association between IL-10 genotypes with gastric cancers specifically the single nucleotide polymorphism of the IL-10 promoter region^[22 & 23].

IL-33 play a role in tumorigenesis by promoting endothelial permeability and angiogenesis^[24]. Recently, IL-33 responds immediately to gastric insult through re-localization and transcriptional changes and may be involved in gastric wound healing and restitution. it was suggested that a class of specialized immune regulators, called *alarmins*, are involved in activating an acute immune response after infection or injury. Alarmins describe a class of multifunctional cytokines released by necrotic cells in response to infection or injury to promote an innate and adaptive immune response. One such cytokine, IL-33 enhances expression of Th2 cytokines and activates multiple immune regulatory cells including group 2 innate lymphoid cells (ILC2) basophils^[11].

We found the correlation between cytokines production (IL- 10 and IL- 33) with the stages of disease when Th1 and Th2 cells work together for development of cancer by immunoregulatory, so the cytokines produced by Th2 down regulate antitumor immunity can be act as tumoricidal macrophages and eosinophils^[25]. This study help patients for diagnostic cancer in early stages and avoid cancer progression by treatment or by surgery removing localized tumor except in the aggressive form which become severe or risk.

Conclusions

According to the results of this study we can conclude that the significant increased in the white blood cells count in both sex at different age groups occur during several cases linking to inflammation and progression of cancer. The increased in the concentrations of IL- 10 and IL- 33 considered to be an impotent and a prognostic immunological parameters for gastric carcinoma.

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