Assessment of IL-10 and IL-33 in Gastric Tumor patients at Babylon Province

Dr. Samah Ahmed Kadhum

Assist. Prof. Medical Microbiology/Head of Department of Clinical Laboratory Sciences/ College of Pharmacy/ University of Babylon, Iraq.

Abstract:

Background:

Gastric cancer remains one of the deadliest diseases with poor prognosis. White blood cell 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. This 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. In 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 cell counts and their different 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%)] **r**espectively 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 an 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 the disease.

Conclusions:

We can conclude that the significant an 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 important and a prognostic immunological parameters for gastric carcinoma.

Keywords: Gastric carcinoma; white blood cell counts; IL- 10; IL- 33 .

INTRODUCTION

Gastric tumor or gastric cancer, also called stomach cancer, that represented by slowly developing through many years by pre-cancerous changes that often occur in the inner lining (mucosa) of the stomach. Tumors are started in different sections of the stomach that may cause different sign and outcomes, also the tumor's location can affect the treatment **[1&2].**

There are many factors probably influence the predisposition of an individual's to gastric tumor and course of it's progression like: gender, age, geography, *Helicobacter pylori* infection, diet, smoking, being overweight or obese. Stomach surgery, pernicious anemia, genetic and familial risk factors, etc**. [3&4].**

Different types of gastric tumors summarized as follows: **Adenocarcinoma**: These tumors can begin in the cells that form the inner most lining of the stomach. **Lymphoma**: These are tumors of the immune system tissues that found in the stomach. **Gastrointestinal stromal t umor (GIST)**: These are rare tumors that found in very early forms of cells in the wall of the stomach called *interstitial cells of Cajal*. **Carcinoid tumor:** These are tumors that started on hormone-making cells of the stomach. Other types of cancer,like squamous cell carcinoma, small cell carc inoma, and leiomyosarcoma, may also be started in the stomach. **[5&6].**

There are four clinical stages of stomach tumor include the following: **Stage 0**: Occur in the inner lining layer of the stomach. **Stage I:** Progression to the second or third layers of the stomach. **Stage II:** Penetration to the more distant lymph nodes, or to all four layers. **Stage III:** Penetration to the fourth layer and either nearby tissues or nearby or m ore distant lymph nodes. **Stage IV:** Tumor has distributed to nearby tissues and more distant lymph nodes, or have metastasized to other organs. **[7].**

Recent researches have provided that *H. pylori* bacteria has the ability to convert some substances in foods into chemicals that cause mutations in the DNA of the cells in the stomach lining layers **[8]** while others foods contain antioxidants that can block substances that damage a cell's DNA. People with stomach tumors have a higher rate of *H. pylori* infection than people without this tumor. *H. pylori* infections are linked to lymphoma of the stomach and with progresses through stages of chronic gastritis, atrophy, intestinal metaplasia, dysplasia, and cancer **[9&10]**.

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. **[11]** The important roles of cytokines can be represented by regulating of immunity and inflammation that caused by bacteria which attractive candidates for being genetic host markers of individual susceptibility to gastric cancer development **[12]**

IL-10 is an immunoregulatory cytokine that regulates differentiation and proliferation of immune cells **[13]**.The dual roles of it can be represented by immunosuppressive and antiangiogenic activity that promoting or inhibiting the effect of tumor development and progression **[14&15]**. 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 **[16].**

Aim: This study was aimed to explain the relationship between some immunological parameters (White blood cells count with differente 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 the 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 ≤)] 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 includes 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 differente 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). 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). 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). In addition to seeing a significant increased (p<0.05) in the number of white blood cells count with their different types in gastric cancer patients in male rather than female compared with control groups as in table (4). Table (5) shows a significant an increased (p<0.05) in the number of white blood cells count with all different 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. Also, we found a significant an increased (p<0.05) in the concentrations of IL-10 and IL-33 in patients of both sexes compared with healthy control groups as in table (6) . 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 (1) *H. pylori* infection in gastric cancer patients

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

Table (2) Risk factors for gastric cancer patients

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

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

Age groups (years)	Male	%	Female	%
≥ 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
61 ≤	3	6.52	4	8.69
Total	23	49.98	23	49.97

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

WBC count 10e3/uL M±SD	Male	P-value	Female	P- value	Control groups	P-value
White cells	18.373±2.106	0.020[a]	16.284±0.958	0.000[b]	6.982±1.072	0.000[c]
Neutophils	12.345±0.656	0.030[a]	11.255±1.243	0.100[b]	5.011±0.341	0.000[c]
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[c]
Monocytes	2.024±1.011	0.020[a]	2.016±2.457	0.020[b]	0.720±1.221	0.000[c]
Lymphocytes	3.381±1.316	0.030[a]	2.218±1.218	0.100[a]	1.985±0.432	0.000[b]

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

WBC count 10e3/uL M±SD	Stages of gastric cancer disease						
	I	p- value	II	p- value	III	p- value	IV
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±10.230
Neutophils	7.025±0.129	0.120[a]	7.198±1.340	0.040[b]	9.223±0.653	0.000[c]	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[c]	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[c]	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[c]	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[c]	4.545±2.324

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

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

IL-10 concentrations (pg/ml) M±SD	Stages of gastric cancer disease						
	I	p-value	II	p-value	III	p-value	IV
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

Il-33 concentrations (pg/ml) M±SD	Stages of gastric cancer disease						
	I	p-value	II	p-value	III	p-value	IV
Male	299.382±8.487	0.050[a]	389.823±9.368	0.000[b]	587.731±9.963	0.030[c]	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 colonized gastric epithelium; thus, spread to the area of intestinal metaplasia with significantly associated with bile reflux and is a feature of autoimmune gastritis [17]. The progression of atrophy and tumor with the presence of *H. pylori* is correlated with the host and bacterial factors that influence they everity of inflammatory response. An increased in inflammation subjects related with colonized *cagA*-positive strains [18&19]. Also, we found that the male patients had a higher prevalence compared with female and this may be associated with several factors such as age, low socioeconomic status, nutritional deficiency and poor hygiene [20]. The highest detection rate of gastric carcinoma was (15.21%) and (13.04%) which recorded in two age groups (41-50) and (51-60) years respectively and this agreement with [20&21]. The excess of gastric cancer disease in positive family history for *H. pylori* infection (39.13%) and in the smoker (28.26%) were agreeing with [22;23&24], and this may be associated with the aging population, changes of lifestyle, physical activities and types of diets. The results indicated an increased in the white blood cell count and this were agreement with many researcher like [25&26], 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 lymphocytes 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[27&28].

IL-33 play a role in tumorigenesis by promoting endothelial permeability and angiogenesis [29]. Recently, IL-33 has responded 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*, is 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 [16].

We found the correlation between cytokine production (IL- 10 and IL- 33) with the stages of disease when Th1 and Th2 cells work together for the development of cancer by immunoregulatory, so the cytokines produced by Th2 down regulate antitumor immunity can be act as tumoricidal macrophages and eosinophils [30]. 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 an increased in the white blood cell count in both sexes 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 importent and a prognostic immunological parameters for gastric carcinoma.

REFERENCES:

[1] Mile, A.; M. Parker; B. Kumar and P. Klark, Gastrointistinal disease In: Clinical medicine. Kluwer Academy Publishers, Dordrecht, U.K., pp.271-280 (2002).

[2] Poonam, S. and A. Chandana, A Review on Anticancer Natural Drugs. International Journal of PharmTech Research, 8(7): 131-141(2015).

[3] American Cancer Society, Global Cancer Facts and Figures. (2007).

[4] Bordbar, G. and N. S. Bolandnazar, Gastroesophageal reflux disease (GERD): prevalence and association with Psychological Disorders among medical sciences students. International Journal of PharmTech Research, 8(7):120-130 (2015).

[5] Sri, G. T.; M. C. Asif; H. Daniel and M. Muntzer, The diagnosis and management of gastric cancer, BMJ, 347:f6367. Clinical Review (2013).

[6] A L - Hasnawy, H. H.; A. T. AL - Hassnawi, and A. M. AL - Ameri, Detection of *Epstin Barr Virus* lmp-1 gene associated with lymphoma. International Journal of PharmTech Research, 9(9):214-219 (2016).

[7] Bing, H.; E. H. Nassim; S. Scott; L. Nancy; B. Robert and M. Aurelia, Gastric cancer: Classification, histology and application of molecular pathology. J Gastrointest Oncol, 3(3):251-261 (2012).

[8]   Sasi, P.; V. Ravichandiran and M. Sumithra, Study of Cancer causing Food Product material Analysis by using UV Spectroscopy. International Journal of PharmTech Research, 8(4): 514-520, (2015).

[9]   Chochi, K.; T. Ichikura; M. Kinoshita; T. Majima; N. Shinomiya; H. Tsujimoto ; T. Kawabata; H. Sugasawa; S. Ono; S. Seki; and H. Mochizuki, *Helicobacter pylori* Augments Growth of Gastric Cancers via the Lipopolysaccharide- Toll -like Receptor 4 Pathway whereas Itsn Lipopolysaccharide Attenuates Antitumor Activities of Human Mononuclear Cells. Clin. Cancer. Res., 14(10): 2909- 2917 (2008).

[10]   Eskandari, A.; A. Mahmoudpour; N. Abolfazli and A. Lafzi, Detection of *Helicobacter pylori* using PCR in dental plaque of patients with and without gastritis. Med. Oral. Patol. Oral. Cir. Bucal., 15 (1):e28-31(2010).

[11]   Dalerba, P.; C. Maccalli and C. Casati, Immunology and immunotherapy of colorectal cancer. Crit. Rev. Oncol. Hematol., 46:33–57 (2003).

[12]   Kunstmann, E.; C. Epplen ; E. Elitok ; M. Harder; S. U. Suerbaum; W. Peitz and J. T. E. Schmiegel, *Helicobacter pylori* infection and polymorphisms in the tumor necrosis factor region. Electrophoresis, 20:1756–61(1999).

[13]   Mocellin, S., Interleukin-10 and the immune response against cancer: a counterpoint. J. Leuko. Biol., 78, 1043–1051 (2005).

[14]   Howell, W. M., Cytokine gene polymorphisms, cancer susceptibility, and prognosis. J. Nutr., 137, 194–199 (2007).

[15]   AL - Awad, A. S., Assessment of Toll like receptors (TLR-8 and TLR-9) in group of patients with different types of tumor in Babylon Provence of Iraq . International Journal of PharmTech Research, 9(11):289-296 (2016).

[16]   Jon, N. BAZ.; V. C. Heather; I. P. Daniel; S. Philip; R. M. Trevelyan; S. G. Andrew and M. J. Louise, IL-33 Is a Stomach Alarmin That Initiates a Skewed Th2 Response to Injury and Infection. CMGH, (1)127-128 (2015).

[17]   Walker, M. M., Is intestinal metaplasia of the stomach reversible? Gut. 52:1–4(2003).

[18]   Asaka, M. and B. A. Dragosics, *Helicobacter pylori* and gastric malignancies. *Helicobacter*., 9(1): 35-41(2004).

[19]   Chong, V. H.; K. C. Lim and N. Rajendran, Prevalence of active *Helicobacter pylori* infection among patients referred for endoscopy in Brunei Darussalam. J. Singapore Med., 49(1): 42-46 (2008).

[20]   Ozen, A.; A. Furman; M. Berber; H. Karatepe; N. Mutlu; H. Saricoban and B. Buyukgebiz, The effect of *Helicobacter pylori* and economic status on growth parameters and leptin , ghrelin and insulin- like growth factor (nIGF)-1 concentrations in children . J. *Helicobacter*, 16: 55-65 (2011).

[21]   AL - Awady, H. K., A study certain physiological changes accompanied with peptic ulcer in Babylon. College of Science ,University of Babylon Ph.D. Thesis, (2001).

[22]   Rajab, H. K., A study of the efficacy of two weeks triple therapy and one week quadruple therapy in peptic ulcer disease. M. Sc thesis in Tikrit university (1994).

[23]   AL - Sulami, A.; H. S. AL - Kiat; L. K. Bakker and H. Hunoon, Primary isolation and detection of *Helicobacter pylori* from dyspeptic patients : a simple, rapid method .La Revue de Sante de la Mediterranee orientale, 14(2):268-276 (2008).

[24]   AL - Bermani, O. K. H., Study of the pathogenesis and pathogenicity of *Helicobacter pylori* in different mammalian animals . College of Science ,University of Babylon, Ph.D. Thesis, (2010).

[25]   Shanker, A.; J. Wang; E. Rochtchina; C. Mimi; R. Kefford and P. Mitchell, Association between circulating white blood cell count and cancer . Arch Intern, 166:188-194 (2006).

[26]   AL - Mudhafar, D. H., Evaluation of some biomarkers in patients with lung cancer. pH. Thesis (2015).

[27]   Devita, F.; M. G. Orditura; C. Galizia; S. Romano; A. Infusino; E. Auriemma, and G. Catalano, Serum interleukin-10 levels in patients with advanced gastrointestinal malignancies. Cancer, 86:1936–43 (1999).

[28]   Stanilov, N. N.; L. Stankova; J. Miteva; T. D. Jovchev and S. Stanilova, Role Of IL-12 P40 and IL-10 in Progression Of Colorectal Cancer. Trakia Journal of Sciences. 8: 132-136 (2010).

[29]   Choi, Y.; H. Choi; J. Min; B. Pyun; Y. Maeng; H. Park; J. Kim; Y. Kim and Y. Kwon, Interleukin-33 induces angiogenesis and vascular permeability through ST2/TRAF6- mediated endothelial nitric oxide production. Blood , 114:3117-26.(2009).

[30]   Jovanovic, I.; G. Radosavljevic; M. Mitrovic; V. Lisnic; A. McKenzie ; N. Arsenijevic; S. Jonjic and M. Lukic, ST2 deletion enhances Innate and Acquired to Murine Mammary Carcinoma. Eur. J. Immunol., 41(7):1902-1912(2011).