RESEARCH ARTICLE

Immunological Study of IFN-γ, ICAM-4, and Vitamin D3 Markers among Gastrointestinal Tumor Patients in Babylon Province, Iraq

Ali Husain Shilib AL-Shimmery[1], Zena Abdul-Ameer Mahdi[2], Hayder Abdul-Amir Makki Al-Hindy[3]*, Raheem Tuama Obayes Al- Mammori[4], Thanaa Abdulmahdi Mokif[5], Hussein O M Al-Dahmoshi[6], Noor S K Al-Khafaji[6]

Abstract

Objective: The current study was conducted to investigate the roles of ICAM-4, IFN-γ, and vitamin D3 markers among benign and malignant gastrointestinal stromal tumors (GISTs). **Methodology:** Eighty-eight participants, admitted to the Babylon GIT Center, Merjan Medical City, Iraq from April to December 2020, were recruited for the study. Blood samples were collected from the participants, who were divided into four groups: malignant GIT tumor (N = 42), benign GIT tumor (N = 29), irritable bowel disease as a positive control (N = 10), and healthy individuals as a negative control (N = 7). Serum ICAM-4, IFN-γ, and vitamin D3 levels were determined using the blood samples. **Results:** The younger males were more affected by malignant GIT tumors at a mean age of 53.39 years than benign GIT tumors, IBD, and healthy individuals. There is also an increase in ICAM-4, IFN-γ, and a decrease in vitamin D3 levels compared to healthy individuals. The vitamin D3 level decreased progressively with age and rose in ICAM-4 with a decrease in vitamin D3 level in patients, increasing the probability of infection with GIT tumor. ICAM-4 levels may grow and increase as interferon levels rise. **Conclusion:** The younger males are more prone to malignant GIT and the serum levels of ICAM-4, vitamin D3, and IFN-γ are high in malignant patients compared with benign GIT tumors and lower than the control.

Keywords: Gastrointestinal stromal tumors- ICAM-4- Vitamin D- IFN-γ- GIT tumor

Introduction

Gastrointestinal stromal tumors (GISTs) are non-epithelial mesenchymal soft tissue sarcomas that can develop in any part of the digestive system. The small intestine and stomach are the most common locations for GISTs. In addition, GISTs are uncommon neoplasms that account for fewer than 2% of all gastrointestinal neoplasms and have a high rate of malignant transformation (Parab et al., 2019). Approximately 10 to 30 % of GISTs develop into cancer. GISTs outside the stomach, on the other hand, are connected to increased malignant potential (Paral et al., 2010). In 18% of cases, particularly those involving smaller digestive tract tumors, GISTs show no symptoms at all. The vast majority of such tumors are detected by coincidence during endoscopies, abdominal CT scans, or surgical procedures for other reasons (Sreide et al., 2016).

Intracellular adhesion molecule-4 (ICAM-4) is an ICAM family member that is expressed in erythroid cells. CD11b/CD18 and CD11a/CD18 were the first reported receptors for ICAM-4 (Ihanus et al., 2007). The interaction of ICAM-1 with integrin CD18 could promote carcinoma cell dispersion (Bai et al., 2015). Cancer cells linked to macrophages enable transcoelomic metastasis via CD11b/CD18-ICAM-1 adhesion (Yin et al., 2016). Prostate and breast cancer risk may be influenced by ICAM-4, ICAM-1, and ICAM-5. Furthermore, ICAM is linked to two prevalent cancers. The first is lymphoma, which is a tumor originating from lymphatic tissues (Al-Mahdi Ghazi et al., 2020), which is linked to ICAM-1 (Liu et al., 2020). The second example is breast cancer, a prevalent location of malignant disease in which ICAM-1 downregulation lowers the metastatic ability of breast carcinoma cell lines. Studies have revealed that tumor cells expressing

*[1]Babylon GIT Center, Babylon, Hilla City, Iraq. [2]College of Pharmacy, Ahl Al Bayt University, Karbala, Iraq. [3]Department of Pharmacology & Toxicology, College of Pharmacy, University of Babylon, Babylon, Iraq. [4]Babylon GIT and Liver Center, Babylon Health Directorate, Hilla, Iraq. [5]Babylon Technical Institute, Al-Furat Al-Awsat Technical University, 51015 Babylon, Iraq. [6]Department of Biology, College of Science, University of Babylon, Hilla, Iraq. *For Correspondence: phar.hayder.abdul@uobabylon.edu.iq*

junctional adhesion molecule-1, ICAM-4, and "leukocyte integrin LFA-1" lead to effective proliferation within the brain parenchyma (Di et al., 2016; Marium et al., 2020).

A multifaceted molecule known as interferon-gamma (IFN-γ) is connected to anti-proliferative, pro-apoptotic, and antitumor processes (Castro et al., 2018). The production of IFN-γ by NK cells two months after treatment with imatinib mesylate is an independent predictive factor for survival in advanced GISTs. Numerous studies have found that exposure to sunlight can increase vitamin D production, which is known to have a role in calcium and phosphate metabolism (Al-Agam et al., 2021). Meanwhile, multiple studies have highlighted the role of vitamin D in gastrointestinal cancers, particularly GISTs (Mahendra et al., 2018). However, few studies have been conducted on ICAM-4, especially in GISTs.

The present research was therefore aimed at investigating the role of ICAM-4, IFN-γ, and vitamin D3 among benign and malignant in GISTs.

Materials and Methods

Ethical approval
Ethical approval for this study was obtained from the Ethical Board of the local health authority. The investigation was conducted following the guidelines stipulated in the 1975 Helsinki Treaty.

Study groups
Eighty-eight patients in the related age group were recruited from different areas of Babylon Province, Iraq for the study. All the patients were admitted to the GIT Center in Merjan Medical City, Babylon, Iraq from April to December 2020. They all provided informed consent before the commencement of the study. After confirming the diagnosis by endoscopy and biopsy section, the participants were grouped into four: 42 malignant GIT tumors, 29 benign GIT tumors, 10 irritable bowel disease as positive controls, and 7 healthy individuals as negative controls.

Immunological analysis of blood samples
Blood samples were collected from all the participants (controls and patients). The blood samples were processed into blood serum. ELISA kits (Elabscience, China) were employed to measure serum levels of IFN-, ICAM-4, and vitamin D_3.

Statistical analysis
Microsoft Office (Excel 10) was used to organize the collected data. Statistical analysis of the data was performed using SPSS (version 25). For univariate analysis, chi-square and one-way analysis of variance (ANOVA) tests were used to determine differences between the levels of the investigated variables. The results were evaluated at a 95% confidence interval (CI).

Results

In the current study, the basic characteristics of the variables were compared between patients with GIT tumors and healthy controls. The average total age was 44.4±18.5 years; however, patients were much older ($p<0.01$) as shown in Table 1.

There were no significant differences in ICAM-4, or INF-γ between the two groups. Vitamin D_3 also showed significantly ($p<0.01$) higher levels among the control groups as presented in Table 1. The serum ICAM-4 levels were higher in patients with malignant GIT tumors compared to benign GIT tumors, positive and negative, or healthy controls, with mean ± SD of 28.7±8.2, 24.9±5.8, 25.8±4.7, and 25.6±5.04, respectively (Table 2). Serum IFN-γ levels were higher in patients with malignant GIT tumors than in benign GIT tumors, but lower in positive and negative controls, with mean ± SD of 46.8±33.5, 39.5±18.1, 48.3±18.6, and 55.2±15.1, respectively (Table 3). As depicted in Table 4, vitamin D_3 levels were lower in patients with malignant GIT tumors compared

Table 1. Basal Characteristics of the GIT Tumor and Control Groups

Characteristics	N	Age (Years)	Male	Female	ICAM-4	Vitamin D3	IFN-γ
Total (Mean ±SD)	88	44.4±18.5	N-56	N-32	26.7±6.6	31.33±11.89	47.4±24.9
GIT tumor N (%)	51	51.1±18.5	28 (54.9)	23 (45.1%)	27.3±7.6	24.01±8.85	44.3±29.1
Control N (37)	37	35.0±13.9	28 (75.7%)	9 (24.3%)	25.7±4.8	41.42±7.71	51.6±17.2
Sig. Value		0.03	> 0.05		> 0.05	0.001	> 0.05

Table 2. T-test ICAM-4 Level among GIT Tumor Patient Groups and Control

Mean±SD ICAM-4 level (pg/ml)				
Malignant GIT tumor	Benign GIT tumor	IBD (Positive control)	Healthy (Negative control)	Sig. Value
28.7 ±8.19	24.96± 5.8	-	-	0.3
28.7 ±8.19	-	25.87± 4.72	-	0.4
28.7 ±8.19	-	-	25.57± 5.04	0.3
-	24.96± 5.8	25.87± 4.72	-	0.7
-	24.96± 5.8	-	25.57± 5.04	0.8
-	-	25.87± 4.72	25.57± 5.04	0.9

Table 3. IFN-γ Level among GIT Tumor Patient and Control Groups

	Mean±SD IFN-γ level (pg/ml)			
Malignant GIT tumor	Benign GIT tumor	IBD (Positive control)	Healthy (Negative control)	Sig. Value
46.85±13.5	39.59±18.14	-	-	0.3
46.85±13.5	-	48.37±18.6	-	0.8
46.85±13.5	-	-	55.29±15..1	0.2
-	39.59±18.14	48.37±18.5	-	0.3
-	39.59±18.14	-	55.29±15.09	0.05
-	-	48.37±18.56	55.29±15.09	0.3

Table 4. Vitamin D$_3$ Level among GIT Tumor Patient and Control Groups

	Mean±SD Vitamin D3 level (pg/ml)			
Malignant GIT tumor	Benign GIT tumor	IBD (+ve control)	Healthy (-ve control)	Sig. Value
20.41± 2.43	30.60±9.72	-	-	0.001
20.41± 2.43	-	45.31± 7.82	-	0.001
20.41± 2.43	-	-	36.84 ±7.80	0.001
-	30.60±9.72	45.31± 7.82	-	0.001
-	30.60±9.72	-	36.84 ±7.80	0.18
-	-	45.31± 7.82	36.84 ±7.80	0.02

to benign GIT tumors, and lower than the positive and negative controls, at mean ± SD of 204.2±24.3, 306.1± 97.2; 453.2±78.2; 368.5±78.0, respectively. The results (Table 5) demonstrate that patients in their fifth decade of life are more likely to develop malignant GIT tumors (mean = 53.39 years), while benign GIT tumors are more likely to arise in their fourth decade (mean = 47 years). Patients with both malignant and benign IBD tumors are older than the control group. There is a negative correlation between vitamin D$_3$ level and age, as well as ICAM-4 level. A direct relationship was obtained between ICAM-4 and IFN-γ. This observation could imply that vitamin D3 levels gradually decreased with age and that elevated ICAM-4 contributed to a decrease in vitamin D3 levels in GIT tumor patients. Increased interferon may cause an increase in ICAM-4 levels.

Discussion

The linearity of IFN-γ, ICAM-4, and vitamin D$_3$

concentrations, as well as optical density, were only investigated. Cell surface glycoprotein receptors known as ICAMs are involved in cell-matrix and cell-cell adhesive contacts (Harjunpää et al., 2019). According to recent findings from the "Dutch GIST-Registry," younger males are more prone to malignant GIT tumors in their fifth decades of life than benign tumors or healthy individuals. In oncology research, the youthful population has gained prominence as a group with unique psychosocial requirements and biomolecular characteristics (de Rojas et al., 2020). Several researchers in this field have focused on the molecular and clinic-pathological characteristics of GISTs in young individuals (Ijzerman et al., 2020).

Previous findings reveal that ICAM-1 and ICAM-4 are both required for eliciting an immunological response (Bui et al., 2020). In comparison to the benign and control groups, the current study results demonstrated a high level of ICAM-4 in patients with GIT tumors. The high level of ICAM-4 observed in GIT cancers is comparable to the high level of ICAM-1 found in gastric and pancreatic

Table 5. The Correlation between All Studied Variables among Patients with GIT Tumor

Variables	Correlation	Age	ICAM-4	Vitamin D$_3$	IFN-γ
Age	Pearson Correlation	1			
	Sig. (2-tailed)	-			
ICAM 4	Pearson Correlation	0.183	1		
	Sig. (2-tailed)	0.088	-		
Vitamin D3	Pearson Correlation	-0.367**	-0.197	1	
	Sig. (2-tailed)	0	0.066	-	
IFN-γ	Pearson Correlation	-0.047	0.252*	0.03	1
	Sig. (2-tailed)	0.661	0.018	0.78	-

*, Correlation is significant at the 0.05 level; **, Correlation is significant at the 0.01 level

tumors of patients with metastases (Gu et al., 2005). ICAM-4, which work with the granulocyte/monocyte-enriched 2 integrin CD11b/CD18 (Mac-1, M2), and may play a similar role to ICAM-1 in the growth and spread of GIT cancers (Ihanus et al., 2007). The fundamental structural similarities and molecules that interact with both ICAM-1 and ICAM-4 may explain the elevated levels of both in GIT malignancies (Harjunpää et al., 2019). According to Ke Wang et al., (2021), the inflammatory microenvironment encourages the adhesion of cancer cells to distant metastatic sites. They concluded that inflammation promotes malignant cell adhesion to brain endothelial vascular cells.

IFN-γ, one of the cytokines with pleiotropic effects, is required for the bulk of adaptive and innate immune responses. Early research in the late twentieth century suggested that IFN-γ has antitumor and anti-infective properties (Zhao et al., 2021). The current study discovered higher serum IFN-γ levels in patients with malignant GIT tumors compared to benign GIT tumors, though lower than in controls. Marth et al., (2004) observed similar findings in their research and reported that a particular microenvironment could impact the neoplastic progression of GIST at various metastatic sites. However, there have been positive outcomes associated with the use of IFN-γ in the treatment of several cancers, including bladder carcinoma, ovarian cancer, and adult T-cell leukemia. Several studies focused on the precise involvement of IFN-γ in the body's reaction to the tumor. According to several studies, IFN-γ is critical for the immune system's cancer surveillance, because it is anti-proliferative and anti-angiogenic, IFN-γ sensitizes tumor cells to death and increases immune activity against malignancies.

In contrast, extensive evidence from ongoing studies associating IFN-γ-based therapies with tumor treatment has had limited effectiveness. Current research suggests that IFN-γ plays a pro-tumorigenic role, primarily through the downregulation of major histocompatibility complex (MHC), the insensitivity of IFN-γ signaling, and the overexpression of "programmed cell death ligand-1" (Castro et al., 2018).

Many cellular pathways that influence cellular proliferation, differentiation, and apoptosis are controlled by vitamin D. Consequently, vitamin D has been linked to tumor frequency, death, and prognosis. Numerous types of research have shown the role of vitamin D in gastrointestinal tract tumors, particularly GISTs (Mahendraet al., 2018). Consistent with these findings, the results from the present study show that vitamin D_3 levels were lower in patients with malignant GIT tumors compared to benign GIT tumors and control groups. Vitamin D has extra-skeletal anti-proliferative effects among various cells, including the breast, colon, prostate, skin, and cancer prevention (Fleet, 2008), mediated by its binding with a specific vitamin D receptor (VDR). VDR helps to regulate vitamin D and calcium levels, as well as inflammation, estrogen pathways, and insulin-like growth factor signaling. Its participation in multiple pathways suggests a possible function for VDR in etiology (Goyalet

al., 2019).

Transforming growth factor-beta (TGF-β) is a pleiotropic cytokine with multicellular activity (Al-Hindy et al., 2021). Evidence recently revealed that the TGF-β signaling pathway is abnormal in GIT cancers, with a complex suppressive and pro-oncogenic impact via serine-threonine receptor kinases (Chen et al., 2016). A potent mitogen is platelet-derived growth factor (PDGF), which is essential for several cellular processes (Mohin et al., 2020). Additionally, it was revealed that GISTs are associated with elevated expression of PDGF and/or its receptors. Multivariate analysis verified that serum PDGF and vitamin D are independent risk factors for cancer. However, another recent study showed the anti-cancer activity of vitamin D, probably via suppression of the expression of VDR (Skrajnowska and Bobrowska-Korczak, 2019).

A positive correlation between ICAM-4 and IFN-γ observed in this study, has not been previously reported in other studies. IFN-γ enhances the expression of ICAMs, the production of inflammatory cytokines, and the antitumor activity of monocyte-macrophage cells. The activation of cellular lipopolysaccharide responses via IFN-γ is referred to as the "priming effect". Beyond cytokine synthesis amplification or monocyte/macrophage adhesion, the specific mechanism is not yet known (Kurihara and Furue, 2013). Cancer immuno-modulation is a process in which both innate and adaptive immune systems, as well as cytokines such as ICAMs, IFN-γ, vitamin D, and others, work together to suppress and/or control tumor growth. Future studies will help to understand the specific signaling mechanisms and interrelationships between distinct bioactive compounds. Nevertheless, this information should be further investigated by a larger cohort of researchers with larger sample size.

In conclusion, according to the findings of this study, younger males are more susceptible to malignant GIT tumors in their fifth decades of life as compared to benign tumors and healthy individuals. Serum ICAM-4 levels were higher in malignant patients compared to healthy controls and benign GIT tumors. Also, serum IFN-γ levels in malignant patients were higher than in benign GIT tumors, but lower than in controls. Serum vitamin D3 levels were lower in malignant patients compared to benign GIT tumors and lower than in the control group.

Author Contribution Statement

Ali Husain Shilib AL-Shimmery, Raheem Tuama Obayes Al- Mammori, Hayder Abdul-Amir Makki Al-Hindy, and Hussein O. M. Al-Dahmoshi: Development of the research idea and results interpretation; Ali Husain Shilib AL-Shimmery and Raheem Tuama Obayes Al- Mammori: Experimental design, execution, and sample collection; Zena Abdul-Ameer Mahdi, Hayder Abdul-Amir Makki Al-Hindy, Hussein O. M. Al-Dahmoshi, and Noor S. K. Al-Khafaji: Statistical analysis and manuscript writing; Hussein O. M. Al-Dahmoshi and Noor S. K. Al-Khafaji: Proofreading and plagiarism check.

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