ORIGINAL ARTICLE

CONTENTS

Interplay between p53 dysfunction and inflammatory cytokines in colorectal cancer pathogenesis

Mayada Hameed Al-Khafaji[1], Sally Imad Hussein[2], Mohammed Ismael Majeed[1]

[1]DEPARTMENT OF MEDICAL LAB TECHNIQUES, COLLEGE OF HEALTH AND MEDICAL TECHNIQUES, GILGAMESH UNIVERSITY, BAGHDAD, IRAQ

[2]BASIC SCIENCE DEPARTMENT, COLLEGE OF DENTISTRY, AL- IRAQIA UNIVERSITY, BAGHDAD, IRAQ

ABSTRACT

Aim: The present research set out to investigate the relationship between F53 and interleukins as well as the possible relationship between p53 with the emergence of malignant lesions, specifically colorectal cancer (CRC).

Materials and Methods: Patients at Baghdad's Medical City Hospital provided the colorectal cancer samples, Forty colorectal patients and forty control samples from healthy individuals between the ages of 35 and 75and cancer specimens made up the eighty samples that were gathered.

Results: This work employed a quantitative real-time polymerase chain reaction (RT-PCR) to accurately assess the expression levels of the p53 gene expression, namely the fold change calculated using the $2^{-}\Delta\Delta Ct$ method, as well as ELISA to detect the levels of IL-6 and IL-8. According to the data, colorectal cancer patients had higher levels of IL-6 and IL-8 (25.8± 7.6 pg/ml, 119.9± 76.7 pg/ml) than control (8.7± 2.3 pg/ml, 43.2± 12.1 pg/ml), instead P53 levels were lower in patients (130.5 ± 65.8 pg/ml) than control (182.4 ± 93.7 pg/ml). The expression of the p53 gene was dramatically reduced in 15 of the 40 colorectal cancer (CRC) samples that were used for RT-PCR. These results point to a possible link between the development of colorectal cancer (CRC) and downregulated p53 expression. With a P value < 0.02, the results indicated a substantial variation in the expression of P53titer among patients and controls.

Conclusions: The cytokines in issue are definitely higher in cancer patients' blood, and due to their critical involvement in the etiology of the disease, they would be appealing targets for novel therapeutics meant to treat colorectal cancer.

KEY WORDS: p53 gene, IL-6, IL-8, colorectal cancer (CRC), real-time polymerase chain reaction, ELISA

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INTRODUCTION

Another of the deadliest and third most prevalent cancers worldwide is colorectal cancer (CRC)., is a serious threat to the world's health. One The illness makes more than just 900,000 deaths every year, and its prevalence shows a concerning increase in groups that are typically thought to be at reduced risk [1]. Increasing epidemiologic impact emphasizes how urgently an extensive comprehension of the pathogenesis of CRC as well as the creation of novel treatment approaches are needed. Despite its ability to combat tumor cells, cellular immunity is essential [2]. T lymphocytes (T-helper 1) produce the lymphokine interleukin-6 (IL-6) and IL-8 which is produced by Th cells. Interleukin-8 (IL-8) is a cytokine that offers significant promise as a predictive and/or prognostic biomarker for cancer. It either autocrinely or paracrinely influences the occurrence of cancers. IL-8 may have a special involvement in breast cancer since the disease is mostly brought on by the production of the estrogen receptor (ER) and the human epidermal growth factors receptor 2 (HER2) [3]. Endothelial cells and macrophages respond by releasing interleukin-8, which was produced by both malignant and stromal cells, respectively. Prior studies have shown that IL-8 accelerates the formation of colorectal cancer malignancies by promoting angiogenesis, metastasis, and invasion by cells [4]. The cellular protein p53 was first discovered to bind to the SV40 large T antigen [5]. A new phase in cancer research is ushered in by this revelation. research that is expected to have a big impact on clinical practice. Stress triggers the transcription factor p53, which controls a large number of genes downstream in order to carry out regulatory tasks in multiple pathways of signaling. Approximately 40–50% of sporadic colorectal cancers have a p53 mutation [6]. The course and result of sporadic CRC are strongly correlated with the presence of p53 mutations. A number of small molecule drugs have been the subject of extensive research in recent years for their potential to restore and reactivate p53 through various ways.

Clinical trials are being conducted on these promising chemicals, which could eventually be authorized for use in the treatment of people with colorectal cancer [7]. The pathophysiology of colorectal cancer involves complicated interactions between inflammatory cytokines and dysfunctional p53. Intestinal cytokine networks are influenced by inflammatory cytokines, which are crucial to the growth of this cancer [8]. Research shows that attempts to counteract abnormal signaling and its role in cancer etiology interact dynamically. They draw attention to the control of cytokines that trigger inflammation and inflammatory/oncogenic pathways. This intricate relationship emphasizes how crucial it is to comprehend these mechanisms in order to create successful treatments. The pathogenesis of colorectal carcinoma and the impact of p53 mutations on clinical outcomes are the main topics of this investigation [9]. Furthermore, this article discusses the recent accomplishments in Reactivating this transcriptional pathway in colorectal cancer (CRC) with p53 modulators may be a promising alternative anti-cancer treatment.

AIM

The present research aims to investigate the regulation of the p53 gene in patients with colorectal cancer. As well as the levels of immunological parameters and the relationships between them.

MATERIALS AND METHODS

ETHICAL APPROVAL

This study protocol approved by Ethical and research committee College of Medical and Health Technologies / Gilgamesh University (number: 343 date: 12/12/2025). Before enrollment in the study, the research concept was verbally explained to the patients and each patient signed consent. The Helsinki Declaration and later amendments for human research were applied for all procedures in this research.

COLLECT THE SAMPLES

To find any genetic variations, blood samples were taken from individuals with colon cancer at the Baghdad's Medical City Hospital from January 2025 to March2025, The patients were between the ages of 35 and 75. The blood's five milliliters taken from each subject were split into two milliliters of in an anticoagulant-free gel tube and left to clot at the temperature of the room. After that, the blood was centrifuged at 2000x for 10 minutes. Serum samples were then kept at -20°C until they were needed for an ELISA test and 3 ml were stored in a tube of EDTA with anticoagulation at -20°C for extraction of RNA. As directed by the kit instructions, the ELISA test (SUNLONG, China) was used to examine the immunological assays for IL-6 and IL-8. TRIzolTM-assisted RNA extraction followed by targeted gene cDNA synthesis. A Korean company called Bioneer supplied the cDNA ready-to-use kit, which was used to create the cDNA for genomic investigation of p53 (Table 1).

CALCULATING THE GENE EXPRESSION FOLD

By implementing these steps, the Levak equation was used to evaluate the fold expression in comparison to the housekeeping gene and control.

Ct (control) - Ct (control over housekeeping) = Δ Ct (control) ΔCt (sample) = Ct (sample) - Ct (housekeeping sample) Δ Ct (control) minus Δ Ct (sample) equals ΔΔ Ct Gene expression foldof = (2^{-}Δ Δ Ct).

CRITERIA FOR INCLUSION AND EXCLUSION

Age over 35 with colorectal cancer confirmed by histology was one of the inclusion requirements readiness to participate in the genomic investigation of p53.

Among the exclusion criteria were further cancers.

PRIMER SEQUENCE INCLUSION

Table 2 lists the sequences, their positions within the gene.

ANALYSIS OF STATISTICS

SPSS version 20, an analytical statistical program, was used to examine the information. The mean ±SD is used to display the data. The data was statistically analyzed using correlation and the t-test. The statistical meaningful level were established at $P < 0.05$.

RESULTS

There were 80 participants in all, 40 of whom were patients and 40 were controls. According to table 3, 10 patients (13%) were in the 35–45 age range, 27 patients (34%) were in the 46–55 age range, 20 patients (25%) were in the 56–65 age range, and 23 patients (28%) were in the 55–75 age range.

When comparing patients with colorectal cancer to the healthy group, the results in table 4 revealed a statistically significant elevation of both IL-6 (25.8± 7.6pg/ml, 8.7± 2.3 pg/ml) and IL-8 (119.9± 76.7p g/m L vs. 43.2± 12.1p g/m L) levels (p=0.003, p=0.001).

Table 5 shows that the p53 titer was lower in colorectal cancer patients than in the unaffected population.

Table 1. The PCR circumstance is revealed

Step	Temperature (°C)	Duration	Cycles
Initial Denaturation	96	3 min	1
Denaturation	94	15 s	40
Annealing	55	45 s	
Extension	74	60 s	

Source: Compiled by the authors of this study

Table 2. The P53 gene primer sequences employed in this investigation

Gene	Primer	Sequence (5′→3′)	Location within sequence (nucleotide)
P53 Gene	Forward	5′-TGCAAAGAGGTGCGTGTAA-3′	702–720
	Reverse	5′-CTGAACCGTCCCTGTAGAT-3′	841–850

Source: Compiled by the authors of this study

Table 3. Age-specific the distribution of colorectal cancer (CRC)

Parameters		N=80	%
Age (years)	35-45	10	13%
	46-55	27	34%
	56-65	20	25%
	55-75	23	28%
Total		80 (100%)	

Source: Compiled by the authors of this study

Table 4. Interleukin means concentrations in patients with colorectal cancer relative to the control group

Interleukins	Patients	Controls	P-value
IL-6(pg/ml)	25.8± 7.6	8.7± 2.3	0.01
IL-8(pg/ml)	119.9± 76.7	43.2± 12.1	0.01

Source: Compiled by the authors of this study

Table 5. P53 titer mean among individuals with colorectal cancer relative to the control group

Parameter	BC Patients	controls	P-value
P53titer	130.5 ± 65.8	182.4 ± 93.7	0.02

Source: Compiled by the authors of this study

This study found that there was a positive correlation between P53 Titer and IL-6 Levels in the Serum of Participants with Colorectal Cancer compared with Control groups (R: 0.073) (Fig. 1).

This study found that there was a positive correlation between P53 Titer and IL-6 Levels in the Serum of Participants with Colorectal Cancer compared with Control groups (R0.1339) (Fig. 2).

DISCUSSION

Recently has been found that many tumor forms, particularly colorectal cancer, have elevated concentrations of IL 8, a cytokine that promotes inflammation with angiogenic properties [10]. It has been discovered that IL 8 acts on cancer cells through its receptors in order to stimulate angiogenesis in vivo as well as migration, invasion, and multiplication in colorectal cancer [11]. According to the current study, individuals with colorectal cancer had a considerably higher IL 8 content than did healthy people. There have been prior reports of increased levels of IL 8 in cancer samples and lines of cells; The findings showed that as contrasted with normal colon tissues, the amounts of IL 8 were higher in CRC tissue. The current study's genome sequencing results revealed seven alterations, one placement, one elimination. and one overexpression in the CRC tissues' IL 8. Tâlvan et al. [12] claim that IL 8 is associated with an increased risk of cancer of the colon. In addition, a higher sensitivity was associated with the heterozygous type of IL 8 to CRC in comparison to control in another earlier investigation including Malaysian patients suffer-

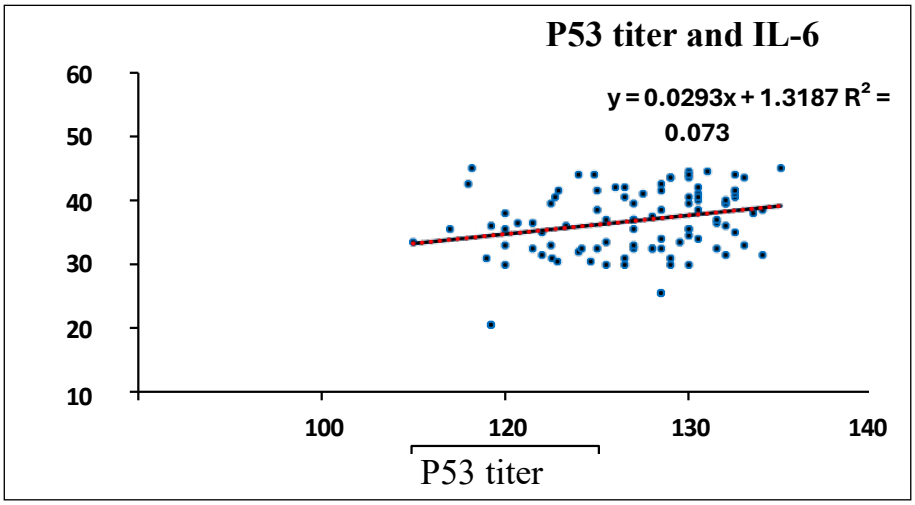

Fig. 1. The Association among P53 titer and IL-6 levels in the serum of participants with colorectal cancer
Source: Compiled by the authors of this study

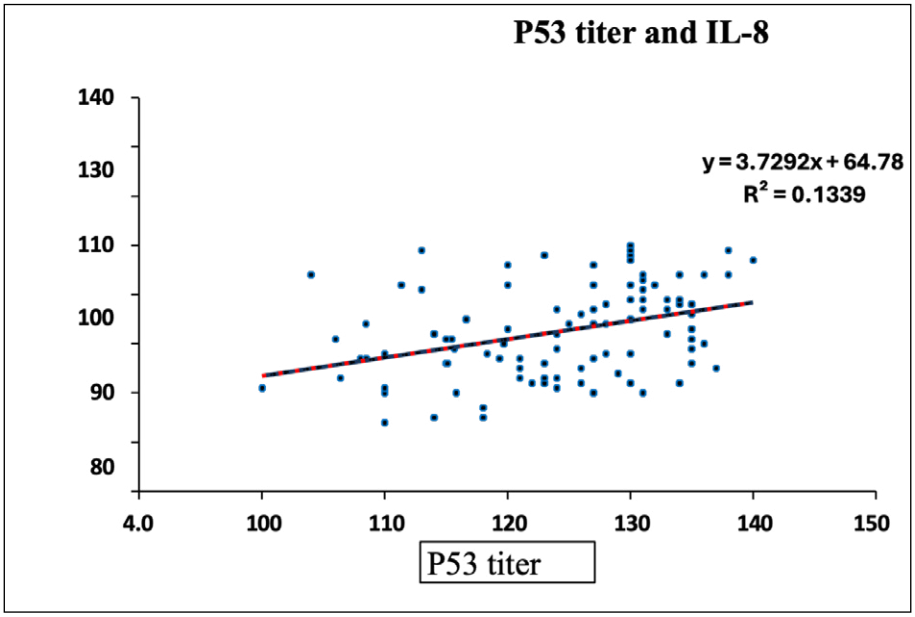

Fig. 2. The association between P53 titer and IL-8 levels in the serum of participants with colorectal cancer
Source: Compiled by the authors of this study

ing from CRC [13]. According to reports, IL 8 is linked to increased promoter activity, which raises IL 8 levels and encourages its proangiogenic actions, ultimately leading to an increase in the proliferation of tumor cells [14]. According to Ghazwan et al., the two groups' p53 levels differed significantly, with the patients' mean being lower than that of the control group [15]. This contrasts with the results of studies by Sofiani et al. [16], who found that, in comparison to control processes, the average circulating blood level of p53-in patients increased significantly.

Similar observations were made by Su et al. [17], who found revealed patients with established tumor phases prior to surgery had significantly greater serum IL-6 levels than healthy controls. The levels were noticeably lower on the 10th day following surgery, though. Furthermore, Pop et al. [18] and Pu et al. [19] made the interesting observation that the levels of IL-6 in the malignant tissue were significantly higher than those in the typical mucosa and that they were closely connected with the levels of IL-6 in the blood serum. The researchers therefore concluded that serum IL-6 concentrations mirror the IL-6 concentrations in the tumor tissue and demonstrate the tumor's capacity to proliferate. The elevated IL-6 levels in the samples of CRC patients undergoing treatment may be caused by the following factors: The CEA has been shown to trigger the production of several cytokines, including IL-6, from the liver's Kupffer cells, which may then promote the growth of tumor cells at metastatic sites [20]. Infected cells from the immune system may also be a source of IL-6. Using RT-PCR analysis, it has been found that p53 andIL-6 proteins are present in colorectal tumor tissue. Nonetheless, this cytokin may be produced by macrophages, which is consistent with other findings that support the idea that inflammatory substances in colorectal cancers originate from monocytosis [21].

CONCLUSIONS

The results showed that colorectal cancer is significantly influenced by IL-6 and IL-8. progression and constitute important biomarker for identification and prediction cancer. The cytokines in question are indeed elevated in cancer patients' blood, and due to their vital roles in the etiology of the disease, they would be desirable targets for innovative therapies intended to treat colorectal cancer. This protein is an excellent option for the treatment of cancer because p53 deficiency is commonly seen in human malignancies.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Mayada Hameed Al-Khafaji
Department of Medical Lab. Techniques,
College of Health and Medical Techniques,
Gilgamesh University,
Baghdad, Iraq
e-mail: mayada.h.mahdi@gu.edu.iq

ORCID AND CONTRIBUTIONSHIP

Mayada Hameed Al-Khafaji: 0009-0001-7484-8296 A B
Sally Imad Hussein: 0009-0003-2106-592X B C D
Mohammed Ismael Majeed: 0009-0004-3833-9082 A C E F

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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