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RESEARCH PAPER

Evaluation of *p53* expression among Colorectal Cancer patients

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A B S T R A C T:

Background and purpose: Colorectal cancer (CRC) is counted as a third most common cancer among men and the second most common among women. Besides that, among cancer-related mortality, the 3rd most common cause of death is due to the CRC worldwide. A tumor suppressor gene *p53* has the main role in regulating cell apoptosis, cell cycle arrest, and cell proliferation. Thus, abnormality in *p53* such as mutation or overexpression has a significant association with human cancer, in which alteration in this gene can be found in more than 50% of different cancer cases. Therefore, our aim in this study is to evaluate the expression level of *p53* gene focusing on its mRNA expression among colorectal cancer patients in Erbil, Kurdistan Region-Iraq.

Material and method: Forty-four pairs colorectal cancer tissues along with their corresponding non-cancerous tissues that were grouped based on the CRC types and patients' clinical features. The expression of the *p53* gene of the samples were evaluated by RT-PCR technique.

Result: results showed that expression of the *p53* gene in colorectal cancer samples was significantly increased (overexpressed) compared to the expression of normal samples (control) (n=44, p=0, 0001).

Conclusion: It might be possible to consider the overexpression of the *p53* gene as a molecular marker for colorectal cancer diagnosis in both men and women. However, for better understanding and confirming our opening findings further analysis are required.

KEY WORDS: Colorectal cancer, *p53*; MTp53; gene expression.
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1.INTRODUCTION :

Colorectal cancer, also called bowel cance, is a sort of cancer which develops in the parts of the large intestine. Both genders are able to develop colorectal cancer. However, the incedences rate is distinct from gender to others. Incedences of CRC in women booked for 9.4% among all cancers which make it the second most common right after breast cancer, while it is considered as the third most common among men by 10.0% globally.

Besides , among cancer-related mortality, the third most common cause of death was due to the CRC (Inamura, 2018). According to the statics of 2008, the estimation regarding the incidence and mortality of CRC showed that, approximately 50,000 death among the whole of 150,000 diagnosed cases (László, 2010). While the death rate was higher in the year of 2012, about 700,000 of the patients faced death due to CRC from a total number of about 1.3 million of newly diagnosed cases. However, the incidences of CRC were expected to be increased by 2030 that can reach the range of 2.2 million or even more within

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a mortality rate in half number of the incidents about 1.1 million (Arnold et al., 2016).
The *p53* gene which is placed at chromosome 17q13.1 encodes the tumor suppressor p53 protein (Huang et al., 2014). When this gene gets mutated, which happens in more than 50% of cancer cases including CRC, it would express a mutant form of p53 (MTp53) which is highly associated with human cancer prognosis (Murata et al., 2013). The role of p53 protein in controlling cell proliferation and apoptosis is well known (Huang et al., 2014). Mutation in *p53* gene leads to loss of controlled cell death ability, which results in unregulated cell growth, accordingly, promoting tumorigenesis (Di Agostino et al., 2013).

This study aims to shed a light on, the expression rate of *p53* among CRC patient tissues, which was examined via RT-PCR. Moreover, to find out whether or not *p53* expression level has a correlation with the progression of tumor and its prognosis among colorectal cancer.

2.MATERIALS AND METHODS

2.1. *Patients and samples:* Overall 44 paired tissue samples (44 tumor tissue and 44 control) were obtained from CRC patients from the Rizgary Hospital in Erbil, Kurdistan Region-Iraq between October 2018 and April 2019. The obtained tissue samples (biopsies) of the CRC were preserved in RNALater in -20 ℃ until RNA analysis.

2.2. *RNA extraction:* RNA was extracted from the obtained colorectal tissue samples using the *ExiPrep*^{TM} Tissue total RNA kit (Bioneer, Korea) following the instruction of its manufacture. Biophotometer (Eppendorf, Germany, model: Biophotometer Plus 6132) was used to determine the quantification and qualification of total extracted RNA.

2.3. *Complementary DNA synthesis:* In order to measure the *p53* gene expression level, the mRNA converted to cDNA using Ipsogen RT Kit (Qiagen, GmbH, Hilden, Germany) following the manufacturer's instructions. Thermal cycling processes Master-cycler pro PCR System (Eppendorf, German) was used to regulate the required condition for the cDNA. Then, the cDNA was amplified by RT-PCR employing the expression primers described in table 1.

2.4. *Expression analysis of p53 gene*: RT-PCR was performed using Master-cycler pro PCR System (Eppendorf, German) with RT^2 SYBR Green ROX FAST Mastermix (Qiagen GmbH, Hilden, Germany) for *p53* expression. The housekeeping gene Glyceraldehyde-3- phosphate dehydrogenase (GAPDH) was used as internal control for normalization (Liu et al., 2014). Moreover, cDNA for *p53* was subjected to RT-PCR using a set of primers designed based on exon–exon junction using online tool primer-BLAST. However, for the GAPDH we used a set of primers which previously described by (Liu et al., 2014) as indicated in Table 1.

MJ Research, AB Applied Biosystem thermal cycler was used to optimize the primer. We have prepared 50µL reaction mixture in PCR tubes containing 2.5 µL cDNA template, 25 µL OnePCR^{TM} master mix (GeneDirex, Korea), 1 µL forward primer, 1 µL reverse primer and 20.5 µL ddH2O. The cycling conditions were set in which initial denaturation at 95 ℃ for 10 min, 35 cycles of denaturation at 95 ℃ for 45 sec, annealing temperatures mentioned in Table 1 for 30 sec and extension at 72 ℃ for 45 sec, and final extension at 72 ℃ for 4 min.

Table 1. The utilized primer sequences, PCR product and optimum annealing temperature.

Primer	Sequence 5'- 3'	PCR product	Annealing temperature	References
p53 gene	F- CTGTCATCTTCTGTCCCTTC	222	57.3	(NCBI, 2018)
	R- TGGAATCAACCCACAGCTGCA			
GAPDH gene	F- GGGTGATGCTGGTGCTGAGTATGT	700	68.1	(Liu et al., 2014)
	R- AAGAATGGGAGTTGCTGTTGAAGTC			

2.5. *Statistical analysis:* Graphpad prism 7 was used to statistically analyze the obtained data. Relative quantification RT-PCR was performed in triplicate. The values of threshold cycle (Lao and Grady) were obtained for *p53* and the Ct of housekeeping gene (GAPDH) was used to normalized the values. ΔCt method was performed in order to calculate relative changes (gene expression with respect to the housekeeping gene) in *p53* expression for both tumor and control samples separately.

In order to evaluate the data of present study, we have used the student's t-test to compare colon and rectum cancerous tissue with normal adjacent tissue of mRNA expression level of P53 gene with considering the normal distribution parameters of our data. The assumed significance value was considered at $p \leq 0.05$. Beside of that descriptive statistical methods (mean, standard deviation, median, minimum, maximum rate and frequency) were considerable.

3.RESULTS

In this study, the expression of the *p53* gene for 44 pair of samples was studied. In order to investigate the expression profiles of *p53* in CRC patients, we have used qRT-PCR to evaluate the *p53* expressions. Moreover, Results revealed that *p53* expression was significantly higher (over-expressed) in tumor tissues than the normal tissue (Figure 1). The level of *p53* gene expression was counted from 44 obtained pairs. It was found out that mRNA expression rate from tumor samples was increased compared with its expression level in control samples (non-cancerous) and it was statistically significant (p=0,0001) based on (T-test; $p \leq 0,05$). The level of mRNA expression is shown in Figure 2. for both control and tumor tissues.

4.DISCUSSION

For the last decades, *p53* gene and its encoded protein have been the most studied diagnostic and prognostic molecular marker among various types of cancer including CRC (van Houten et al., 2002, Yamashita et al., 2003). The *p53* gene which locates on chromosome 17q13.1 encodes the tumor suppressor p53 protein or named as wild type p53 (WTp53) (Huang et al., 2014). The *p53* gene is considered as a key part in mediating cell response for several sorts of stresses via inducing or suppressing those genes which are involved in apoptosis, DNA repair, cell cycle arrest, senescence and angiogenesis (Khailany et al., 2017). Numerous studies have reported *p53* overexpression among CRC cases. El-Mahdani et al. (1997) stated that over-expression of *p53* gene occurs frequently in colorectal tumors. Moreover, the *p53* gene mutation as well as the accumulation of p53 protein which is resulted in overexpression of its gene are common genetic feature in CRC (Akshatha et al., 2016). The mutation or loss of the *p53* gene can be identified in more than 50% of all different human cancers (Lee et al., 2013). The expression of mutated *p53* is results in MTp53 and it causes problems in cell cycle which leads to uncontrolled cell growth that promotes tumorigenesis. We intended to determine possible relationship of mRNA expression level and *p53* gene in CRC patients.

Several experiences that are new in cancer biology is depending on the investigation of gene expression examination (Huang et al., 2014, Khailany et al., 2017). This is because of examination of mRNA expression can be considered as an extremely valuable tool in the detection of cancer malignancy, as well as the classification of cancer as a prediction of disease resultant. In our study, the level of mRNA expression of *p53* gene was significantly increased (Up-regulated) as indicated in Figure 1 and 2. Similar results have been founded by (Akshatha et al., 2016, Smith et al., 1996) who also reported the *p53* overexpression in CRC.

Losing functionality of tumor suppressor genes which they normally inhibit tumor development, such as *p53* is well known event (Huang et al., 2014). Furthermore, in the case of *p53* gene the event not only stops with losing its function but also it works oppositely by stimulating tumorgenicity process (Theodoropoulos et al., 2009). For instance, the tumorigenicity osteosarcomas and pre-B cells fibroblasts has increased due to up-regulation of MTp53 (Huang et al., 2014). Additionally, in the case of human T-cell acute lymphoblastic leukemia with mutant p53 alleles which naturally occurs with stable expression have been found, in which increases tissue invasiveness and tumor formation (Theodoropoulos et al., 2009).

In the recent cancer-related studies, the main focus is on the alterations in the gene expression regulation that is not related to the DNA sequence changes and are known as the epigenetic alterations such as DNA methylation and alteration of microRNA expression that have main effect on mRNA expression (Goel and Boland, 2012). However, the effect of epigenetic modification cannot be considered as changes only in gene expressions that happen through modified interactions between the mRNAs or DNA regulator portions (Goel and Boland, 2012). This usually occurs through modifications in gene promoters, in splicing of transcripts or in the stability of transcripts. Although epigenetic alteration has been extremely useful as a diagnostic marker of colorectal cancer, it will not be enough and only marker to be depend on in diagnosis of CRC (Goel and Boland, 2012).

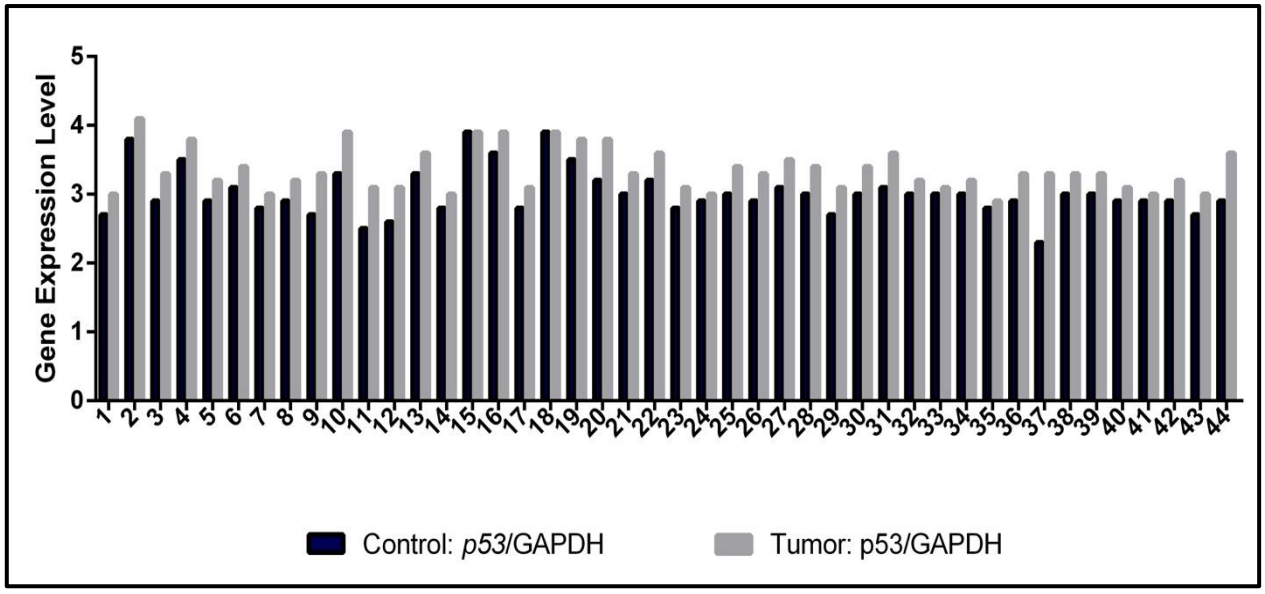

Figure 1. The level of mRNA expression of both normal and tumor tissue among CRC according to *p53*/GAPDH. The level of mRNA expression in 43 samples in tumor tissues increased compared with normal tissues.

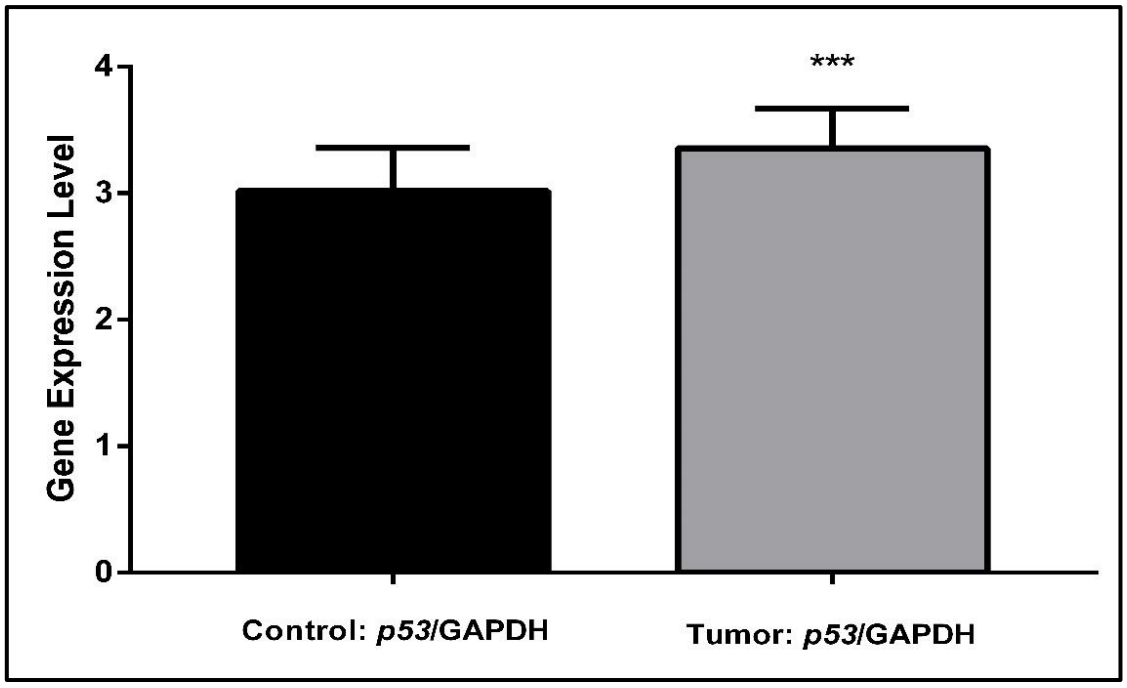

Figure 2. The statistical result of mRNA expression level of *p53*/GAPDH in both normal and tumor tissue samples among CRC patients.

5.CONCLUSIONS

All in all, it can be concluded that there was a significant relationship between overexpression of *p53* gene in tumor tissue and CRC occurrence which evaluated by RT-PCR. Thus, it can be stated that increased mRNA expression of *p53* gene could be a risk factor for colorectal cancer development. Despite of having evidences about relationship between CRC and *p53* gene further molecular investigations are essential including epigenetics and MicroRNA analysis in order to fully understand the correlation between colorectal cancer and molecular biomarkers.

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