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ARAŞTIRMA/RESEARCH

Gene expression of P53 and adipoq as diagnostic markers for colorectal cancer

Kolorektal kanser tanı belirteçleri olarak adipoq ve P53 gen ekspresyonları

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Abstract

Purpose: Colorectal cancer is the most frequent cause of death and had high mortality rate in Western world. It is from complex, variable and patient- specific interaction between genetic, epigenetic and environmental factors. In the present study, we aimed to investigate the contribution of gene expression of the P53 and Adipoq, both genes to the risk of colorectal cancer. P53 gene is a tumor suppressor gene, encoded protein of P53 is a transcription factor and its pivotal role in maintaining genomic stability. Adipoq gene codes adiponectin.

Material and Methods: Total RNA were extracted from paired tumor and normal tissues of 32 colorectal cancer patients. The mRNA expression level of P53 and Adipoq were measured employing semi- quantitative reverse transcription- polymerase chain reaction (RT- PCR).

Results: The mRNA expression level of P53 in colorectal cancer was significantly increased according to normal samples (over-expressed). However, the mRNA expression level of Adipoq in colorectal cancer was significantly reduced according to normal samples (down-regulated).

Conclusion: In current study, our data suggest those reduced mRNA expression of the Adipoq and increased mRNA expression of P53 might be useful molecular diagnostic markers for colorectal cancer patients. In order to understand the investigation between colorectal cancer and diagnostic biomarker; further analysis is necessary.

Key words: Colorectal cancer, P53, adipoq.

Öz

Amaç: Kolorektal kanser Batı dünyasında çok sık karşılaşılan ve ölüm oranı yüksek bir hastalıktır. Genetik, epigenetik ve çevresel faktörler arasında, hasta spesifik bir interaksiyon vardır, dolayısı ile çok çeşitli ve komplekstir. Bu çalışmada P53 ve Adipoq genlerinin ekspresyon seviyelerinin, kolorektal kanser riski üzerine etkisini incelemeyi amaçladık. P53 geni tümör süpressör bir gen olup, bir transkripsiyon faktörü olan P53 proteinini kodlar, genomik stabilitenin devamlılığında çok önemlidir. Adipoq geni adiponektini kodlar.

Gereç ve Yönetim: Otuz iki kolorektal kanserli hastanın, tümör ve normal dokusundan RNA izolasyonu yapıldı. P53 ve Adipoq mRNA ekspresyon seviyeleri semi-quantitative reverse transkripsiyon-polimeraz zincir reaksiyonu (RT-PCR) yöntemi ile belirlendi.

Bulgular: Kolorektal kanserde, P53 ekspresyon seviyesinin normal dokuya göre önemli oranda artış gösterdiği tesbit edilmiştir (aşırı ekspresyon). Fakat, Adipoq ekspresyon seviyesinin, kolorektal kanserinde normal dokuya oranla önemli derecede azaldığı belirlenmiştir (azalmış ekspresyon).

Sonuç: Yaptığımız bu çalışmada elde ettiğimiz veriler, Adipoq gen ekspresyonu seviyesinin azaldığı, P53 geni ekspresyon seviyesinin ise artış gösterdiğine dair olup, bu genlerin kolorektal kanserli hastalarda teşhis markırı olarak kullanılabileceğini önermektedir. Kolorektal kanser ve teşhis biyomarkırları arasındaki ilişkiyi anlamak için, daha fazla çalışma yapılması gerekir.

Anahtar kelimeler: Kolorektal kanser, P53, adipoq.

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common gastrointestinal tumors worldwide1. Epidemiological researches have demonstrated that some risks and interactions between environmental and genetic factors may play important roles in the CRC pathogenesis[1]. The risk of progress CRC increases with age, and over 90% of sporadic CRCs occur in individuals over the age of 50[2].

Many researchers have reflected that CRC is associated with a number of risk factors; such as smoking, family history or inflammatory bowel disease, obesity, alcohol consumption and diet[3]. CRC and their common precursors adenomatous colon polyps, are heterogeneous and complex diseases[5]. The CRC develops through a stepwise accumulation of epigenetic modification and genetic defects, leading to the transformation of normal colonic mucosa into invasive cancer[2].

The number of genes linked to colon cancer are very limit[5]. Many researches have observed the relationship between single nucleotide polymorphisms (SNPs) in the Adipoq gene and CRC risk in diverse populations[5]. The Adipoq gene coding for adiponectin, also it is known as AMP1 gene, it located on the long (q) arm of chromosome 3 (3q27), consists of two introns and three exons[6]. Last decade, researchers interested to investigate the correlation between the biological regulation and genetic role of adipocytokines7. As a unique member of the adipocytokine family, adiponectin, which is an adipose-specific protein, appears to have an antiatherogenic, anti-inflammatory and anti-diabetic effect[7,8,9]. Adiponectin is an adipose tissue-specific cytokine, which plays an important role in the regulation of insulin sensitivity, lipid metabolism and glucose homeostasis[10]. The reduce of plasmaadiponectin levels are associated with type 2 diabetes, obesity and coronary artery disease[11]. In addition, decreased adiponectin expression levels are also reported in colon cancer patients, proposing the role of adiponectin in the CRC pathogenesis[4].

The p53 is the most frequently altered gene in human cancers and more than 50% of human cancers contain p53 mutations, which is guardian of the genome[12,13]. Its most crucial normal function is likely to direct cell cycle arrest at the G1 or G2 phase of the cell cycle after certain types of DNA damage and to stimulate apoptosis when the impairment is too severe[14]. Human P53 gene, consists of 11 exons, which is codes a protein with 393 amino acids[15]. Several prior studies have revealed gene and protein expression of p53 via reverse transcriptase- PCR and immunohistochemistry techniques[15].

Since Adipoq and P53 are mostly altered genes leading to CRC development. In the present study aimed to evaluate the possible association between mRNA expression level of P53 and Adipoq, and colorectal cancer by monitoring RT-PCR analysis.

MATERIAL AND METHODS

Sample collection

The samples were collected from the Rizgary hospital in Erbil, Iraq. A total of 64 samples were analyzed. The study included 32 paired normal and tumor samples of patients that were grouped according to the colorectal cancer types and the clinical features of the patients, including gender and average of age (Table 1). The colon tissue samples kept at -80°C until further analysis. The study was approved by the local ethics committee and was conducted in accordance with the guidelines of the declaration of Helsinki.

Table 1. The number of patient samples, according to types of colorectal cancer, and genders.

Cancer types	Male/pair	Female/pair	Total/pair
Colon Adenocarcinoma	8	8	16
Colon cancer metastasis	4	5	9
Rectal cancer	2	1	3
Appendix cancer	1	1	2
Anal canal cancer	1	1	2

RNA extraction

RNA samples from colon biopsy tissue were obtained by using ExiPrep™ Tissue total RNA extraction kit (Bioneer, Korea) according to the manufacture's instruction. Quantification and qualification of total RNA concentration was performed by using NanoDrop (ND- 1000, USA). Samples with (A260 – A320) / (A280 – A320) ratios less than 1.7 and/or yields less than 0,5 µg total RNA were excluded from subsequent analysis.

Complementary DNA synthesis

Complementary DNA (cDNA) is synthesized from a mRNA template in a reaction promoted by the enzymes DNA polymerase and reverse transcriptase. In this study, cDNA made by using AccuPower Cyclescript RT PreMix kit (Bioneer, Korea). The work area was cleaned by 70% (v/v) ethanol and filter tips were used in all steps Variable amount of total RNA were utilized for each sample since quality and quantity of total RNA are not equal.

RT- PCR and expression analysis

The cDNAs were amplified by semi- quantitative reverse transcriptase- PCR with two pairs of specific primer (Table 2), were designed by SDSC workbench online primer design program, and a pair of primer of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (housekeeping gene) were employed to normalization of p53 and Adipoq gene expressions data[16]. PCR reaction and condition was performed by using MJ Research, AB Applied Biosystem thermal cycler (Eppendorf, Germany). 50 µL reaction mixture was prepared in PCR tubes containing 1.75 µL cDNA template, 25 µL OnePCR™ master mix (GeneDirex, Korea), 1 µL forward primer, 1 µL reverse primer and 22.25 µL ddH2O. The cycling conditions comprised of initial denaturation at 94˚C for 5 min, 35 cycles of denaturation at 94˚C for 45 sec, annealing temperatures in Table 1 for 30 sec and extension at 72˚C for 45 sec, and final extension at 72˚C for 4 min.

Expression alteration were discriminated using agarose gel electrophoresis (2%) in the presence of ethidum bromid. The image of agarose gel was captured and quantitated mRNA expression level by image J software program ((version 1.46r, downloaded from http://imagej.nih.gov/ij)[17].

Statistical analysis

The mRNA expression level of Adipoq and P53 genes in CRC were compared with normal adjacent mucosa employing Wilcoxon signed rank test, significance was assumed for values $p \leq 0.05$. The statistical tests were made by employing SPSS software (V.16).

Table 2. Sequence, site, PCR product size and annealing temperature of utilized primers.

Primer	Sequence	Site	PCR product (bp)	Annealing temperature (˚C)
p53 gene Forward	5'-ACA CGC TTC CCT GGA TTG G-3'	168-186	466	58
Reverse	5'-GGT CTT GGC CAG TTG GCA A-3'	616-634		
Adipoq gene Forward	5'-CTGTTGCTGGGAGCTGTTCTA-3'	139- 159	210	53.9
Reverse	5'-TGGATCTCCTTTCTCACCCT-3'	325- 348		
GAPDH gene Forward	5'-GGTCCACCACCCTGTTGCTGT-3'	Random	456	59.4
Reverse	5'-AGACCACAGTCGATGCCATCAC-3'	region		

RESULTS

Expression analysis

In the current study, the mRNA expression levle of P53 and Adipoq were separated by 2% agarose gel electrophoresis and staining by ethidium bromide. Expression level of P53 and Adipoq genes were obtained from 32 pairs; tumor and normal samples. Figure 1 show expression alteration of Adipoq and P53 geneThe Adipoq expression of 32 CRC patients were obtained from normal tissues and tumors. The expression level of Adipoq of each patients were

different, the comparison between normal controls and tumors is indicated in Figure 2. The mRNA expression level of 30 tumors from 32cancerpatients according to normal samples were decreased. The expression level of P53 of each patients were different, as shown in Figure 3. The mRNA expression level of 30 tumors from 32 cancer patients compared to normal controls were increased. Quantity of expression level of P53 tumor samples were statistically significant increased according to mRNA expression of normal samples (P53: p= 0,00). However, quantity of expression level ofAdipoq tumor samples were decreased according to normal samples (Adipoq: p= 0,00) found by (Wilcoxon signed rank test; $p < 0,05$) (Figure 4).

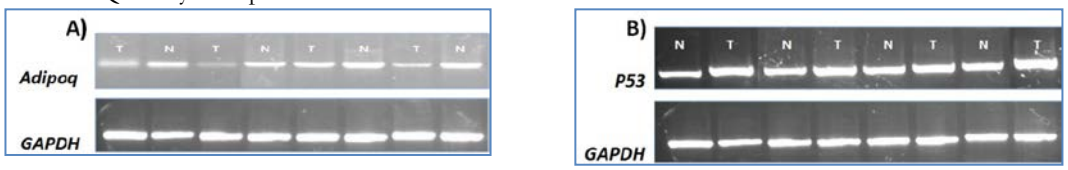

Figure 1. The mRNA expression results of 2% agarose gel electrophoresis staining with ethidium bromide: A) Adipoq and GAPDH genes expression in normal controls and tumor samples in CRC: B) P53 and GAPDH genes expression in normal controls and tumor samples in CRC.
The base pairs of the mRNA fragments; P53= 466 , Adipoq= 210 and GAPDH= 456. N= normal sample: T= tumor sample.

DISCUSSION

A vital goal in nowadays investigation is the discovery of new molecular markers that can detect colorectal tumors in early stages[18]. Lately, molecular genetics researches have prolonged the chance for testing new potential biomarkers as only a few markers can be suggested for practical use in clinic[18]. Expression analysis studies of gene have issued in many new insights in cancer biology and expression analysis of mRNA is turning out to be a very useful tool for cancer diagnosis, cancer classification and disease resultant prediction[18].

Adiponectin encoded via Adipoq gene, that is entirely released by the adipose tissue. In the last years, the relationships between risk of cancer and adiponectin, and its genetic alterations have been widely studied[5]. Although many researches have investigated the association between cancer and adiponectin, the relationship between cancer and adiponectin receptors remains largely unexplored[19].

Conducted by An et al., (2001) comprising of 2,632 cases of colon tumor and 2,753 normal controls showed that gene expression level of adiponectin was significantly decrease in patients according to normal people, which indicated the protective role of adiponectin in colon tumor development[5]. Also Naoto et al., (2009) indicated that decreased gene expression of the Adipop is correlated with venous invasion in CRC[19]. In our study based reverse transcription-PCR, Adipoq gene was significantly reduced in CRC patients. This observation was similar with another study that showed decrease mRNA expression level of Adipoq.

Over the past two decades, p53 has been of the most studied presumptive prognostic and diagnostic markers in CRC[5]. Analysis of the p53 mRNA in CRC by quantitative RT-PCR provides a rapid and sensitive method for discriminating between tumours overexpressing mRNA of p53 with or without p53 gene mutation[5]. Mahdani et al., (1999) reported that mRNA of P53 gene over-expression is a frequent event in colorectal tumours[20]. Several previously published series have supported the deleterious effect of p53 overexpression on CRC prognosis[21].

In our study, the mRNA expression level of P53 gene was increased (over-expressed) as shown in Figure 3 and 4, it was statistically significant. Similarly, Mahdani et al., (1999) and Amal et al., (2010) also showed increased expression of p53 in patients with colorectal carcinoma[20]. The p53 has been the most investigated of all potential markers that may have prognostic or predictive value for patients with CRC[21].

It is well known that tumor suppressors are officially outlined by a loss of function participated in blocking tumor development[21]. Consistent with this definition, by nature happening mutants of p53 are generally defective in sequence-specific DNA binding and consequently do not promote the appropriate target genes, cause cell cycle arrest, or mediate cell death[21]. However, in contrast to a classical tumor suppressor, the p53 gene mutation

leads not only to a loss of function but also to a gain of function that induces the tumorigenicity of various p53-null cell types[21]. Up-regulation of mutant p53 in osteosarcomas and pre-B cells fibroblasts dramatically increase the tumorigenicity of these cells independent of a transdominant negative mechanism[21]. In addition, stable expression of naturally occurring mutant p53 alleles in human T-cell acute lymphoblastic leukemia cells raise tissue invasiveness and increase tumor formation[21].

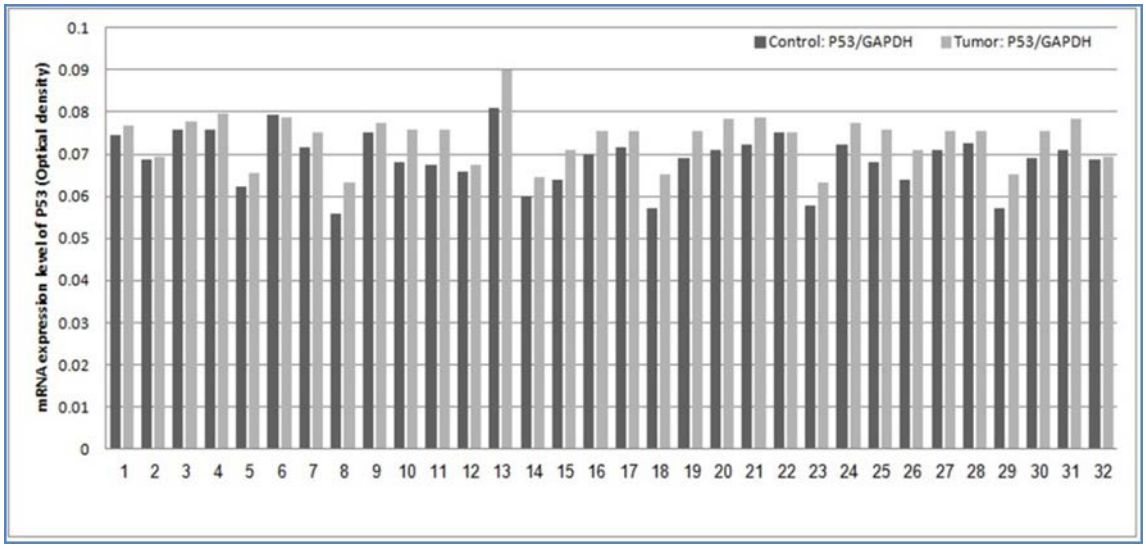

Figure 2. The mRNA expression level of each normal controls and tumors according to P53/GAPDH gene.

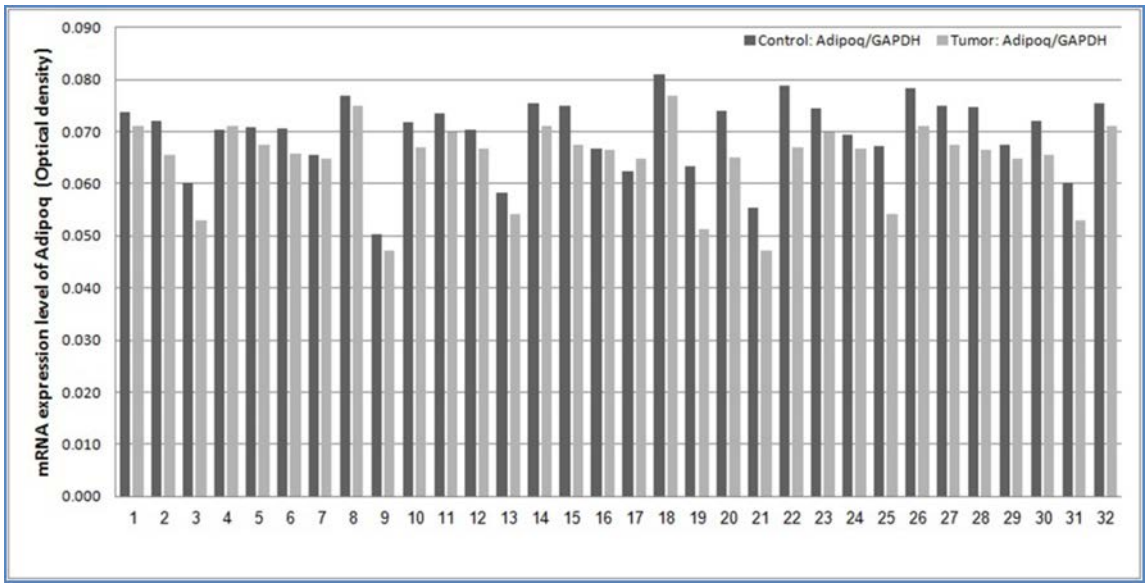

Figure 3. The mRNA expression level of each normal controls and tumors according to Adipoq/GAPDH gene.

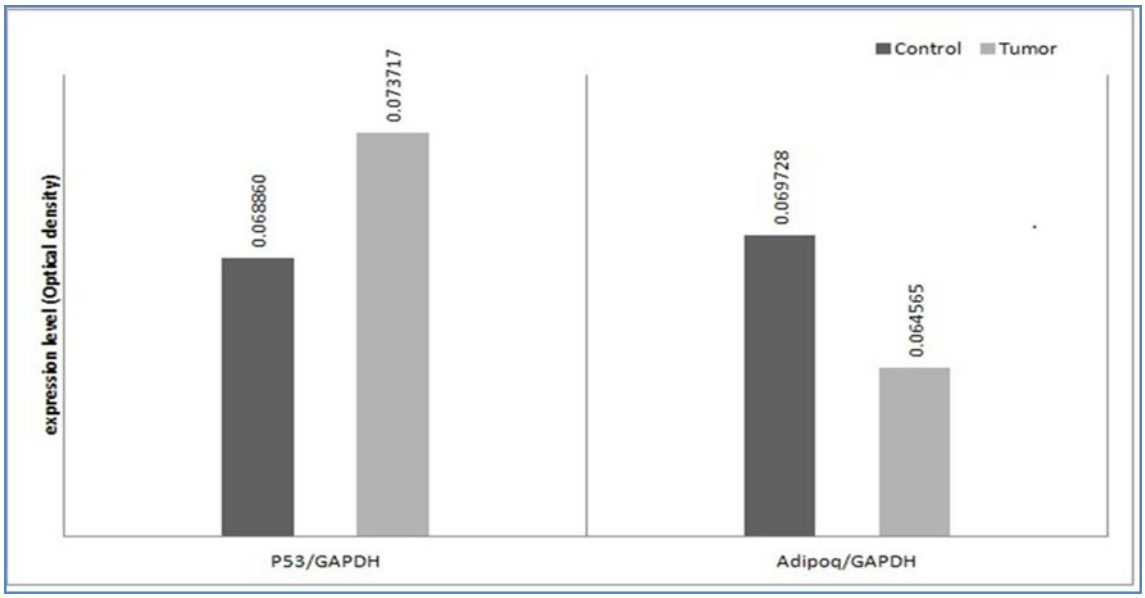

Figure 4. Statistical results of the mRNA expression level of P53/GAPDH, and Adipoq/GAPDH genes in both normal and tumor samples.

Recently in cancer study, observation has been focused on modifications in the gene expression regulation that do not contribute a change in the sequence of DNA of the cell[22]. These are named to as epigenetic alterations, and the most prominent involves changes in DNA methylation. However, epigenetic can be viewed more broadly to include all of the changes in gene expressions that occur through modified interactions between the mRNAs or regulatory portions of DNA that are not directly caused by a change in the sequence of DNA[22]. This usually occurs through alterations in the gene promoters, alterations in the splicing of transcripts or modification in the stability of transcripts[22]. Although epigenetic alteration has been extremely useful as a diagnostic marker of CRC[22].

In conclusion, the mRNA expression level of Adipoq gene was significantly down-regulated and the mRNA expression level of P53 gene was significantly over-expressed. Understanding the molecular mechanisms involved in the pathogenesis of RCC patients. Large and well-designed studies are needed to known the role of Adipoq and P53 genes in CRC.

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