Detection of *BRAF* Gene in Some Iraqi Bowel Inflammation and Colorectal Cancer Patients

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Abstract

The impacts of the inflammatory process on neoplasia development were observed in many cancer, it has a great role in the etiology, development and progression of invasive colorectal tumors. This study was designed to investigate the *BRAF* mutation and assist the clinicopathological parameter in some Iraqi bowel inflammation and colorectal cancer patients. Thirty patients were enrolled in this study (15 suffering bowel inflammation and 15 having colorectal cancer). *BRAF gene* was screened for the presence of mutations using PCR technique and direct sequencing. .The results revealed no *BRAF* mutation in position 1799 for exon fifteen in both samples of bowel inflammation and colorectal cancer. These results were confirmed previous articles regarding low rate of *BRAF* gene mutation in Asian countries. These results indicate the possibility of colorectal patients treatment with monoclonal antibodies. Several heterogeneity mutations were found in 3 out of 30 patients (10%) including transition two mutations G>A and one mutation was transversion mutation A>T in different sites as silent mutations.

Key words : BRAFV600E, bowel inflammation , colorectal , Iraq , heterogeneity.

Introduction

Several important molecular alterations that have important function in etiology and progression of colorectal have been widely studied by researcher such as mutations of the *KRAS* and *PIK3* genes in addition to methylation process like in *P16* , *MLH1* and *BNIP* genes also were explored [1] . The other main gene is *BRAF* which has more than 6890 associated publications listed in PubMed data base but has a little data in Iraq.Oncoprotein BRAF is a serine / threonine kinase protein encoded by *BRAF* proto oncogene , it has essential role downstream of *KRAS* in signaling transduction mitogen activated protein kinase (MAPK) pathway [2,3]. The impact of *BRAF* gene is promoting cell growth and proliferation in addition to differentaition by activating MAPK pathway also affect on important cellular processes like cell migration , apoptosis and survival [4].

Mutations in *BRAF* oncogene were reported, more than 80% of mutation in position 1799 for exon fifteen resulting thymine to adenine transversion causing substitution within codon 600 which were represented by valine to glutamic acid change at protein level leading to constitutive kinase activity[5,6]. Rad and colleagues showed that transformation of colonic epithelia into traditional serrated adenomas and sessile serrated adenomas polyps has been resulted from *BRAF* mutation, they suggested that *BRAF* mutation act as an early event in development of CRC [7]. Many reports refer association of *BRAF* mutation and prognostic status of colorectal cancer regardless microsatelite instability, they found *BRAF* mutation conferred a worse overall survival in patients with advance stage of colon cancer [8,9].The function of *BRAF* mutation status as a prophetic molecular marker is less apparent. Although colorectal cancer have rapidly increased in Iraq but there are little references about *BRAF* mutation status in colorectal patients. The aim of this study was to detect the *BRAF* mutations and assist the clinicopathological parameter in some Iraqi bowel inflammation and colorectal cancer patients.

Materials and Methods

Tissue samples

Tissues biopsies samples were obtained from thirty Iraqi patients (15 suffering bowel inflammation and 15 having colorectal cancer) who were attending the Gastroenterology and Hepatology Diseases Center in Baghdad between October, 2014 and July, 2015. The Diagnosis and selection of patients were assessed under the supervision of pathologist committee.

DNA Extraction

Genomic DNA was extracted from 5-8-µm-thick paraffin sections containing a portion of tumor tissue using the QIAamp DNA Mini kit (Qiagen), Hilden, Germany.

PCR and Sequencin

More than 240 bp DNA fragment of the exon fifteen of BRAF gene was targeted to amplify using forward primer, (5'-atgcttgctctgataggaaaatga and reveres primer , 5'-agcagcatctcagggcca) as described by Kim with colleagues (10). Each 25µl PCR reaction mixture for *BRAF* gene amplification contained 9.5 µl of genomic DNA, 12.5 µl of master mix and 1.5 µM of each primer PCR, amplifications were performed in an Applied Biosystem 96 thermocycle . Amplifications reaction was done using a 15-min initial denaturation at 95 ℃, followed by 35 cycles of 30 sec at 94 ℃, 30 sec at 59 ℃, and 30 sec at 72 ℃, and 10 min final extension at 72 ℃ . PCR products separated in 1.5% agarose gel after staining with ethidium bromide. Molecular marker (Kapa universal ladder) was also loaded in sperate well.

All DNA templates were processed for direct sequencing of single strand PCR reaction using Big Dye Terminator by Microgene (Korea).

Results

Patients and disease

Thirty patients with colorectal disease were investigated, including 15 samples with bowel inflammation and 15 samples with CRC. The mean age of the colorectal patients was 50 years with average 38-62 years , (53.3% (8 cases) were females and 46.6% (7 cases) were males). The severity of the tumor was shown to be moderately differentiated adenocarcinoma 60%(9 cases) ,well differentiated adenocarcinoma well 23.3% (5 cases) and 6.6% (1 case) represented the poor differentiated adenocarcinoma . Fifteen patients with bowel inflammation with maen age 48 years including 53.3% (8 cases) males and 46.6% (7 cases) females. The other specific character of samples are shown in tables (1and 2).

PCR and sequence analysis

Amplified exon fifteen of the *BRAF gene* by PCR technique and screened for the presence of mutations in 30 colorectal disease patients. The results showed that the product size was more than 240 bp Figure (1). To investigate the mutation status of colorectal patients for *BRAF* V600E, direct sequencing was performed after amplified exon 15 for *BRAF* gene in colorectal patient tissues of all cases, the sequences results were compared with NCBI data base (NCBI accession number HM459603). Our analyses illustrated that none of patients had *BRAF* V600E mutation. Several heterogeneity mutations were found in 3 out of 30 patients (10%). Transition mutations (G>A) were shown in 2 out of 3 mutations (66.6%) including one mutation (171495 G>A) in inflammation sample, other mutations were (171506 A>T) and (171537 G>A) in tumor samples. Figure (2) shows reprehensive sequence of colorectal patient tissues.

Discussion

In our previous study of some molecular markers alterations we found 33% mutations in codon 1213 of exon 2 of *KRAS* gene while mutational hot spots (X9) and (X20) of *PIK3* gene were not described in any of 58 Iraqi patients with colorectal cancer (11). Present article highlighted of *BRAF* gene mutations which act essential role in (MAPK) signal transuding pathway, it concerned in the malignant transformation of colorectal precursor. *BRAF* gene is also of interest as being a prospective prognostic and predictive sign in patients with colorectal malignancy (12)

Our study aims to detect *BRAF* gene mutations in inflammatory bowel tissue and compare it with colorectal cancer tissues in Iraqi patients as there evidence is that inflammatory processes have a great role in the etiology, development and progression of invasive colorectal tumors (13). The impacts of the inflammatory process on neoplasia development were observed in many cancer such as breast, ovarian, endometrial, cervical, prostate and colon tumors, it is though that the innate immune system cells have a role in the microenvironment of tumors by secreting pro inflammatory cytokines and chemokines in addition to growth factors and reactive oxygen species that may lead to DNA damage (14). The bowel inflammation process may have important role in the pathogenesis of colorectal cancer, especially in its promotion (15). Balkwill and Mantovani investigated the effects of inflammation in colorectal, they found relationship between cancer and chronic inflammation (16). It is well known that *BRAF* mutation is associated with tumor development through the serous serrated pathway, rather than the classic adenoma-carcinoma pathway (17, 18).

This study indicated that *BRAF* gene mutations were not detected in any of analyzed bowel inflammation tissues neither colorectal cancer tissue, several heterogeneity mutations were identified in *BRAF* gene 10% (3 out of 30) patients including transition mutations (2G>A) in both inflammation and tumor samples of colorectal , in addition to transversion mutation (A>T) that showed in tumor colorectal sample. These results are consistent with recent study that examined *BRAF* gene with 70 colorectal cancer samples and 10th healthy

participates from Iraqi population (19).The present data confirm previous articles regarding low rate of *BRAF* gene mutation in neighbors countries, a study on the frequency of *KRAS* and *BRAF* gene mutations from 242 showed that *BRAF* gene mutations were not present in any of the metastatic colorectal cancer tissues which analyzed in Iranian population (20) also in Saudi Arabia 2.5% (19/757) (21) and Turkish population 2% (1/50) (22). In the other Asian countries, a total of eighty five CRC patients were enrolled in study of Molaei and his colleagues (2016), their results showed no V600E mutation in the *BRAF* gene in stage I and II of CRC patients (23). Other article referred no *BRAF* mutations were found in stage III colon cancer. *BRAF*, *KRAS* and *NRAS* mutations do not have major prognostic value in stage II and III colon malignancy (24) , *BRAF* mutation in Taiwan (0%) (25) and Japan in low rate (4.7%) (26).

Other study was done to investigate the prevalence of several genes including BRAF mutations among 45 patients from Asian population with metastatic colorectal cancer, no mutations were detected in four codons 439, 459, 600 and 601 of the *BRAF* gene . The results of current study inconsistent to reported rate of 19.8% and 21.8% in Netherlands and American people respectively (27) . Yi with colleagues from China published of the 77 *BRAF* V600E mutations, 64.9% (50/77) were found in serrated lesions fifteen percent in serrated adenomas and 36.9% in hyperplasic polyps and 7.1% of other types of colorectal cancer precursor lesions (28).A very low incidence of inflammation bowel and colorectal cancer with *BRAF* mutations reflected in this study and overall Asian population may be due to many reasons such as different ethnic populations , role of environmental influences like diet, smoking and other unknown factors. Furthermore varied underlying genetic predisposition to BRAF-mutated tumors, different methods used to detect gene mutation (21,29) .

In present paper could not associate the V600E mutation with clinicopathological character because no cases with this mutation, this relation was seen with lymph-node metastasis, T4 tumors, mucinous histology, high grade, and right-side location causing specific high risk clinicopathologic parameter (30,31). in addition to right-side primary and female gender have high chance to harbor a *BRAF* mutant (32,33).

In conclusion : this study indicates that no *BRAF* mutation was found in both samples of bowel inflammation and colorectal cancer instead that three heterogeneity mutations were seen in different sites of *BRAF* gene . Other studies are required with large size of cases to investigate *BRAF* gene in Iraqi patients to supports these results.

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Table (1) Distribution of patients with colorectal cancer

Characterization	Patients no. (%)
All patients	15 (100)
Mean age	50 years
> 50	7(46.6)
< 50	8(53.33)
Gender	
Male	7(46.6)
Female	8(53.33)
Site of tumor	
Colon	7(46.6)
Rectum	5(33.33)
Rectosigmoidal	3(20)
Differentiation	
Moderately	9(60)
Well	5(23.33)
Poorly	1(6.6)

Table (2) Distribution of patients with bowel inflammation

Characterization	Patients no. (%)
All patients	15 (100)
Mean age	48 years
> 50	6(40)
< 50	9(60)
Gender	
Male	8(53.3)
Female	7(46.6)

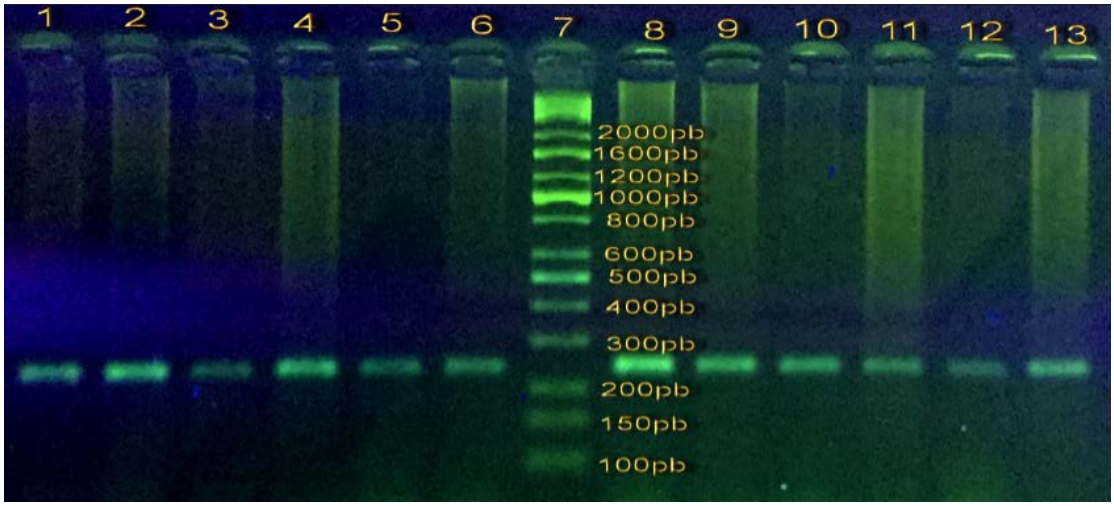

Figure (1) PCR products for exon 15 of *BRAF* gene for DNA samples of colorectal disease on 1.5 % agarose gel. Molecular markers, line (7) and lines (1-6,8-13) DNA of patient samples.

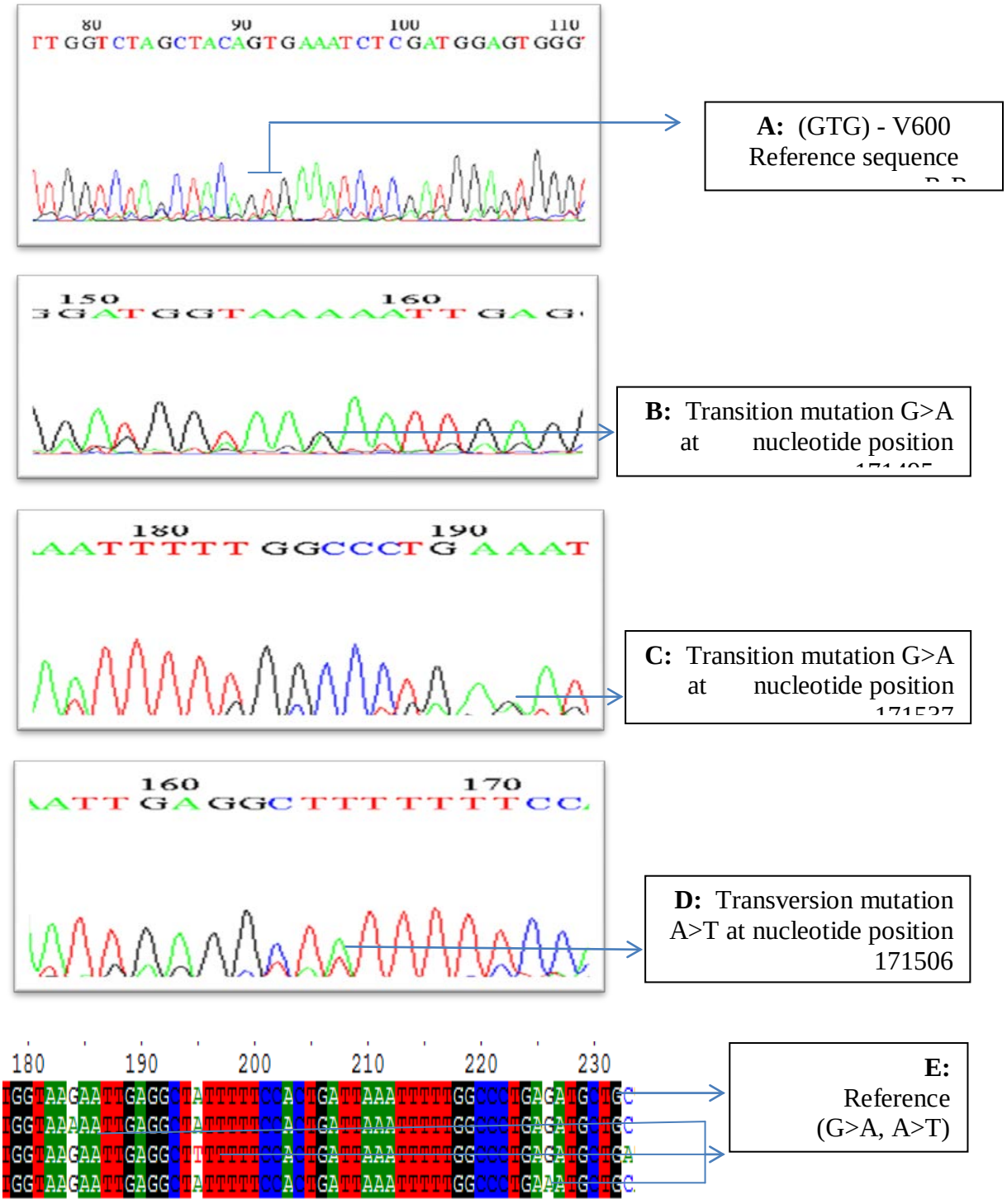

Figure (2) Sequencing alignment A: Reference sequence of the *BRAF* gene (B, C and D) heterogeneity mutations in different site of *BRAF* gene screening in colorectal patients, E: sequences of reference and heterogeneity mutations.

الكشف عن جين *BRAF* في بعض المصابين العراقيين بالتهاب الامعاء واورام القولون والمستقيم

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استلم في: 30/ حزيران/2016 , قبل في: 12 /كانون الاول/2016

الخلاصة

هناك تأثيرات للعملية الالتهابية بحدوث الاورام Neopplasia تم ملاحظتها في العديد من الاورام , اذ ان العملية الالتهابية تملك دوراً كبيراً في نشوء وتطور وتفهقر اورام القولون والمستقيم الغازية .
صممت الدراسة الكشف عن طفرة جين *BRAF* وتقييم علاقتها مع الصفات السريرية لبعض المصابين العراقيين بالتهاب الامعاء واورام القولون والمستقيم . تضمنت الدراسة 30 مريضا (15 مصاباً بالتهاب الامعاء و15يعانون من اورام القولون والمستقيم) . تم االكشف عن الطفرات باستعمال تقانة تفاعل البلمرة المتسلسل PCR ومن ثم تحديد التتابع النيوكليوتيدي Sequencing. اظهرت النتائج عدم وجود طفرة في موقع الشفرة 1799 من اكسون 15(التي يعرف بانها عرضة للتطفير في كثير من الاورام) في اي من عينات التهاب الامعاء واورام القولون والمستقيم وهذا يتماشى مع البحوث السابقة بخصوص النسبة الواطئة لطفرات *BRAF* gene في البلدان الاسيوية مما يتيح احتمالية علاج مرضى اورام القولون والمستقيم بالاجسام المضادة وحيدة النسيلة . اظهرت الدراسة وجود عدة طفرات Heterogeneity mutations في 3 من 30 عينة مرضية (10%) اثنين منها G>A طفرات انتقالية وطفرة واحدة عكسية A>T في مواقع مختلفة من اكسون 15 وهي طفرات صامتة .

الكلمات المفتاحية : جين *BRAF* , التهاب الامعاء , القولون والمستقيم ,العراق وعدم التجانس .