Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.03.041

Kras Mutations and Egfr Expression as Prognostic and Predictive Factors in Cancer Patients

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Submitted: 15 November 2022; Accepted: 05 December 2022; Published: 18 January 2023

ABSTRACT

Cell proliferation is largely believed to be under the control of the epidermal growth factor receptor (EGFR), a member of the tyrosine kinase family of proteins. The existence of point mutations in the K-ras proto-oncogene, a member of the RAS gene family, is an essential determinant in cancer development. These alterations, which are involved in the control of signal transmission across the plasma membrane, play a significant role in cancer development. Mutations in the K-ras gene are frequently discovered in pancreatic, lung, colon, and stomach cancer patients. K-ras mutations are most commonly seen in codons 12 and 13, with codon 61 being the least prevalent. This study examined the prognostic and predictive effect of EGFR expression and K-ras mutations in gastric cancer patients. The study group consisted of patients with stomach cancer who were followed up at Baghdad Teaching Hospital between March 2018 and April 2022. They were all diagnosed using paraffin blocks and slides. The Department of Pathology records at Baghdad Medical College in Iraq were searched for information on these patients' demographic and pathological characteristics and their survival rates. The immunohistochemistry method was utilised to examine the epidermal growth factor receptor (EGFR) expression in 62 patients' stomach tissues. The stomach tissues were paraffin-fixed, and sections were cut from them. Mutagenic PCR and RFLP methods were used to investigate the occurrence of K-ras oncogene codon 12- and 13-point mutations. DNA was isolated from paraffin tissue samples acquired from EGFR-positive people. In 62 gastric cancer patients, the genotype frequencies of the K-ras gene codon 12 were determined to be 93.62 %, 6.38 %, and 0%, respectively. The genotype frequencies of the gene codon 13 were found to be 73.08 %, 26.02 %, and 0%, respectively. From previous studies it was clear that patients with wild-type K-ras polymorphism alleles responded favourably to Cetuximab and had their growth reduced. Individuals with these genotypes are also prevalent in our patient population. We have reason to believe that polymorphism research will be advantageous, notably in the treatment of personalised cancer care.

Keywords: *Cancer patients, Baghdad, K-ras, polymorphism, genotype, PCR.*

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INTRODUCTION

In many parts of the world, stomach cancer is the leading cause of death. An estimated 870,000 new cases and 650,000 deaths annually make gastric cancer the second most frequent malignancy after lung cancer [1]. GLOBOCAN 2018 data shows that stomach cancer is the third leading cause of death for women and the second major cause of death for men. Among men, the rate is 9.6 per 100,000, whereas among women it is 5.7. [2-3]. Men are twice as likely as women to have stomach cancer. The average age of diagnosis is 56 years. Our country's average age is ten years younger than that of Western civilizations [3].

Environmental, dietary, and familial variables all have a role in gastric cancer. The pathogenesis remains unknown. Molecular oncology has advanced our understanding of tumour cell transformation and features in recent years. Two cancer-causing molecules are EGFR and K-ras. Extracellular impulses help cells develop and survive. To improve signal transmission networks, growth factors connect to cell surface receptors. EGFR (erbB-1, HER) is a tyrosine kinase receptor. HER2 (c-neu, erbB2), erbB-3 (HER3), and erbB-4 are all members of the HER family (HER4). EGFR promotes tissue development and homeostasis[4].

K-ras mutations account for 90% of human cancer ras mutations. K-ras mutations are most common at codons 12, 13, 59, and 61. All studies agree: K-ras mutation in the 12th codon is prevalent in adenocarcinomas. K-ras is a proto-oncogene on the short arm of chromosome 12 (12p12.1) that has a function in intracellular signal transduction [5]. The K-ras oncogene encodes a 21-kDa protein with GTPase activity. 2 isoforms (4A and 4B isoforms). In the K-ras gene, which has four exons, the fourth exon is alternatively spliced; the 4A transcript has 189 amino acids; the 4B transcript has 188 amino acids. 25 amino acids at the carboxyl end differentiate them. 4B is 10-20 times more expressed than 4A. [6]. H-ras, K-ras, and N-ras are the most prevalent oncogenes in human cancers. They act as a molecular switch in cell development, govern signal transmission across the plasma membrane, and bind guanosine nucleosides [7-8]. 15–25% of human malignancies and 25% of epithelial cancers include mutant RAS proteins that stimulate cell proliferation. ras mutations at codons 12 and 13 are common, and 90% occur in K-ras. This study aims to determine the prognostic and predictive value of EGFR expression and K-ras mutation in gastric cancer patients.

MATERIAL AND METHODS

Chemicals and Solutions Used

All chemical and reagents used in this study purchased from Sigma Aldrich-USA, with 98 to 99% purity, AR quality. All solutions prepared with double distilled water.

Working group

The study group consisted of 62 gastric cancer patients who were followed up and treated in Baghdad Teaching Hospital (BTH), Medical Oncology, Medical Faculty Pathology Department between 2018-2022. The patient group consisted of 38 men (M) and 24 women (F). The step of the research work study related to EGFR expression was done at BTH, and the step related to K-ras codon 12 and 13 mutation was done in BTH laboratory.

Examination Methods Used

Selection and Storage Conditions of Paraffin Tissue Samples

Paraffin blocks and slides of patients determined to be suitable by examining their pathology reports were removed from the archive. The slides of the cases were revaluated and marked under the light microscope. Paraffin blocks belonging to the marked slides were selected by matching. Paraffin blocks were stored at 4˚C until DNA was isolated.

Methods Of Egfr Expression

Immunohistochemistry Method

Positively charged slides contain 3-micron paraffin-embedded tissue slices. The slides were incubated overnight at 56°C. The next day, the slides were deparaffinized in xylene, 100% alcohol, and 90% alcohol before being rehydrated in distilled water for 5 minutes. The slides were heated in 10% EDTA for 15 minutes to recover the antigens. The pressure cooker top was raised and the slides were removed after 20 minutes. The slides were cleaned with distilled water, dried, and the tissues were drawn with a PAP-PEN pen. Endogenous peroxidase was inhibited by dripping hydrogen peroxide on tissues for 10 minutes.

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After cleaning the slides in distilled water, they were placed in PBS for 5 minutes. Protease 25 was administered for 5 minutes after PBS. 7.4 PBS kept the slides for 5 minutes beyond this interval. Hydrogen Peroxide Block (Protein Block) was dripped for 5 minutes after PBS. The slides were then washed in PBS and incubated for 2 hours with a 1/50 concentration of EGFR (Lab Vision) primary antibody. After that, the tissues were treated for 30 minutes with Biotinylated Goat Anti-Polyvalent. After that, the slides were washed in PBS-filled tubs. The slides were treated with Large Volume Streptavidin Peroxidase after 30 minutes. After that, the slides were washed in PBS-filled tubs. After 5 minutes, the slides were treated with AEC chromogen. When the tissues went red, the slides were cleaned in tap water. The slides were sealed with Clear Mounting Solution after counterstaining and were ready for light microscopy investigation.

Modalities For Identifying The K-Ras Codon 12 & K-Ras Codon 13 Mutation

Patient tissue samples preserved in paraffin were used to extract genomic DNA. DNA samples were counted after they were collected. At first, DNA sample quality was verified using β Globin polymerase chain reaction. K-ras codon 12 and 13 gene products were amplified to a total of 162 and 159 base pairs (bp) using mutagenic polymerase chain reaction (Mutagenic PCR), respectively. The duplicated products were used in a restriction fragment length polymorphism (RFLP) analysis to determine the genotypes.

DNA Isolation

DNA isolation used High Pure PCR Template Preparation Kit (ROCHE). Slices from paraffin blocks treated in 10% formaldehyde were placed in Eppendorf tubes after 30 minutes in xylene. Tissues were cleaned for 10 seconds in 100%, 80%, 60%, and 40% ethanol solutions and water. Then, 200 µl of lysis solution and 40 µl of proteinase K were pipetted over the tissue. Overnight, 37°C was added to Eppendorf tubes. After overnight incubation, 20 µl of proteinase K was added, and the eppendorf tubes were baked at 55˚C for another hour or two. After 10 minutes, the melted tissue fragments were re-incubated at 70˚C for another 10 minutes. After incubation, add 100 µl isopropanol. The Eppendorf sample was then centrifuged at 14,000 rpm for 1 minute. After centrifugation, we replaced the collection tube with a new one, added 500 µl of removal buffer, and spun the mixture for 1 minute at 14000 rpm. The mixture was then centrifuged at 14000 rpm for 1 minute in a new tube with 500 µl of wash buffer. After centrifugation, we discarded the collecting tube and used a new one, then added 500 µl of wash buffer and spun it for 1 minute at 14000 rpm. Next, a fresh tube was centrifuged at 14000 rpm for 10 seconds without solution. The filter tube was transferred to an Eppendorf tube, 200 µl of elution buffer was added, and the mixture was incubated for 5 minutes to attach the DNA. Discarding the collection tube.

Measuring the Amount of DNA

The concentration and purity of the isolated DNA were measured with the Picodrop Microliter Spectrophotometer Version 3.0. 1µl of DNA sample in each measurement and Elution as a blank buffer solution (Roche) was used. The concentration of the isolated DNA samples was determined from the optical density (OD) at 260 nm, and the purity was determined from the OD ratio at 260 nm/280 nm.

There is an established relationship between 1 optical density (OD) and 50 □g/ml for double-stranded DNA molecules. Both the concentration and purity of double-stranded DNA can be calculated using the following formulas (formula a and b, respectively):

DNA concentration (ng/µl) = A260 x Dilution rate x 50 (a)

DNA purity = A260 x Dilution rate x 50 / A280 x Dilution rate x 50 (b)

Preparation of Primers

The β Globin PCR primers applied to control the quality of the isolated DNA samples and the forward and reverse primers used for the determination of K-ras codon 12 and K-ras codon 13 genotypes were dissolved with DNase, RNase-free double distilled water (100 pmol/µl). Then, stocks (10pmol/µl) were prepared and stored at -20˚C. A mismatched upstream primer was used for K-ras codon 12 to recognize the BstNI cut region, and a mismatched downstream primer for K-ras codon 13 was used to recognize the HaeIII cut region.

Mismatched forward primer used for K-ras codon 12 and modified by extending the back primer 9 bp (46). The sequence of primers used for K-ras codon 13 was found in Chang et al. (2009).

The primers used in the β Globin control PCR in this study and the sequences of the oligonucleotide primers used to determine the K-ras codon 12 and K-ras codon 13 genotypes are given below (Table 1). In Table 1, the underlined bases in primer sequence K-ras codon 12 and 13 are mismatch.

TABLE 1: Sequence of Oligonucleotide Primers

	forward primary	**back primer**
Globin primers	5'-CCAAGAAGAGAACTTGTGGGTATTT -3'	5'- CTTCATCCAATTCACCCGCC - 3'
Kras codon 12 Primers	5'-ACTGATATACTGACTTTGGATGTGGCT-3'	5'-CATCAAATGTATTCCTGCCAAAGCAGTAAATGGAC-3'
Kras codon 13 Primers	5'-TATTTGTACTATAGTGTATGGTGGAGGTAA-3'	5'- GTCGTCAAGTCACTCTAGTGCAGCTG-3'

K-ras codon 12 Amplification by Mutagenic Polymerase Chain Reaction

In order to control the quality of the DNA samples of the patients, first of all, the β Globin gene was amplified by PCR. After detecting that they were amplified, the region including the 12th codon of the K-ras gene was amplified. Standard 25 µl PCR mix was prepared in 0.5 ml thin-walled PCR tubes.

K-ras codon 13 Amplification by Mutagenic Polymerase Chain Reaction

In order to control the quality of the DNA samples of the patients, first of all, the β Globin gene was amplified by PCR. After detecting that they are amplified, codon 13 of the K-ras gene region was duplicated. Standard 25 µl PCR mix (excluding primers) was prepared in 0.5 ml thin-walled PCR tubes.

Mutagenic PCR Conditions

Mutagenic PCR conditions were created to amplify the desired polymorphic region. β Conditions of globin control PCR, K-ras codon 12 and K-ras codon 13 mutagenic PCR.

Imaging of Mutagenic PCR Products in Horizontal Gel Electrophoresis

The concentration of the agarose gel to be used in gel electrophoresis varies according to the size of the piece to be separated. In this study, β Globin PCR products applied as control PCR were run on 2% agarose gels, amplified K-ras codon 12 and K-ras codon 13 gene products were run on 2% gels, RFLP products were run on 4% gels, and the gels were ultraviolet. Examined under (UV) light. For 2% gel; 1 g of agarose was dissolved in 50 ml of 1X TAE reconstituted from 50X master stock. When it reached the temperature to be held by hand, 2 µl of ethidium bromide (EtBr) was added and mixed thoroughly. The combs to form the wells were placed in the cassette and the gel was poured onto it. After the gel had frozen, the combs were carefully removed. 10 µl of PCR products were mixed with 2 µl of loading buffer and loaded into the wells. The DNA in the gel was run for 30 minutes in 1X TAE buffer at 90 V using the Thermo Scientific electrophoresis system. The sizes of the PCR products were determined by comparing the PUK8 and the 25 bp DNA molecular weight ladder standard.

Restriction Fragment Length Polymorphism (RFLP) Analysis

162 bp PCR products amplified to determine K-ras codon 12 genotypes were cut using BstN1 (Mva1) enzyme. 10 µl of PCR product was incubated with 1 µl enzyme in a water bath at 37°C for 1 night. In order to determine K-ras codon 13 genotypes, 159 bp PCR products amplified were cut using HaeIII (BsuR1) enzyme. 10 µl of PCR product was incubated with 1 µl enzyme in a water bath at 37°C for 1 night.

Statistical Analysis

The statistical work was done in SPSS (Version: 17.0), a package application for statistical analysis. The study's biostatistical analysis used mean, SD, frequency, and percentage ages to describe variables; the chi-square test was used to compare those values. When comparing the means of continuous variables that follow a normal distribution, the t-test was applied. The survival analysis was conducted using the log-rank test and the Kaplan-Meier technique. In addition, we employed logistic regression, the entry technique, and Fisher's exact test. In making the inferences, a P value of less than 0.05 was used as the threshold for significance.

RESULTS

This study used paraffin blocks belonging to 62 gastric cancer patients who were followed up and treated in Faculty of Medicine Pathology Department, Baghdad teaching hospital, Baghdad, Iraq between March 2018 and April 2022. Our gastric cancer patient group consists of 24 women with an average age of 59.33 and 38 men with an average age of 61.08. While 52 (83.87%) of 62 gastric cancer patients in this study had lymph node metastases, 10 (16.13%) had no lymph node metastasis. According to the TNM classification, 10 (16.13%) for N0, 29 (46.77%) for N1, 18 (29.03%) for N2, and 5 for N3. (8.07%) were detected. When the distribution of tumors according to their anatomical locations was examined, 9 of the tumors (14.52%) originated from the cardia, 15 (24.19%) from the corpus, 31 (50%) from the antrum, and 1 from the other (1.61%). Diffuse involvement was detected in 6 patients (9.68%). Macroscopically, 42 (67.74%) of the cases were infiltrating, 14 (22.58%) ulcerous, and 6 (9.68%) fungal. Among the histological types, adenocarcinomas were the most common with 48.39% (30/62). In addition, 25.81% (16/62) were evaluated as undifferentiated carcinoma, 11.29% (7/62) signet ring cell carcinoma, 14.51 (9/62) as other. Histological types evaluated as other were neuroendocrine carcinoma 8.06% (5/62) and mucinous carcinoma 6.45% (4/62). All of the patients were evaluated as operable and 50% had total gastrectomy and 50% subtotal gastrectomy. Considering the distribution of patients according to the stages, 5 (8.06%) patients in stage I, 26 patients in stage II (41.94%), 26 patients in stage III (41.94%), and 5 patients in stage IV (8.06%) were found. According to Logistic Regression and log-rank test, a highly significant correlation was found between survival and stage (p=0.001). The patient with a high stage dies early. According to the Logistic Regression test, a significant relationship was also found between survival and age (p=0.048). As age increases, the chance of survival decreases. According to the Enter method, the microscopic type is a clinically important parameter for survival (p=0.068). Our study group's 5-year survival rates of gastric cancer patients according to the stages with the Kaplan Meier Chart (Figure 1). The median survival according to the Kaplan-Meier method is 20 months (SD:7.252).

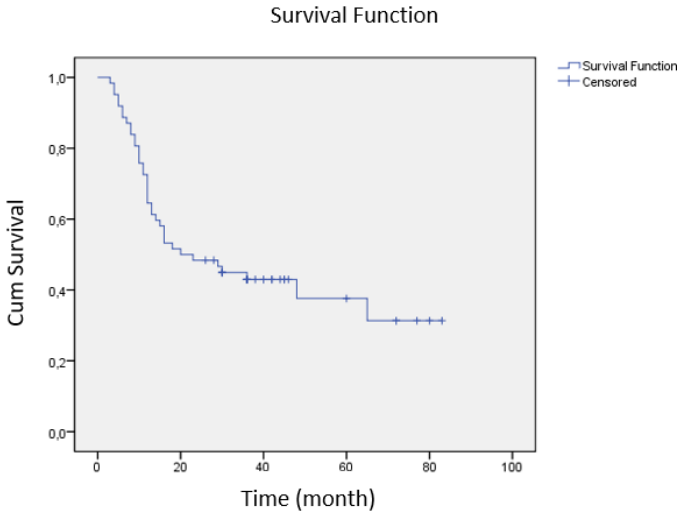

FIGURE 1: Kaplan-Meier Chart

Immunohistochemistry Results

Sections stained with immunohistochemical antibodies were evaluated under the light microscope (Figure 2). It was observed that EGFR showed membranous and cytoplasmic staining. While performing the statistical evaluation, the patients were divided into 2 groups, positive and negative, and the results were evaluated. It was observed that 48 of the 62 patients showed positive EGFR expression (77.42%) and 14 (22.58%) negative staining.

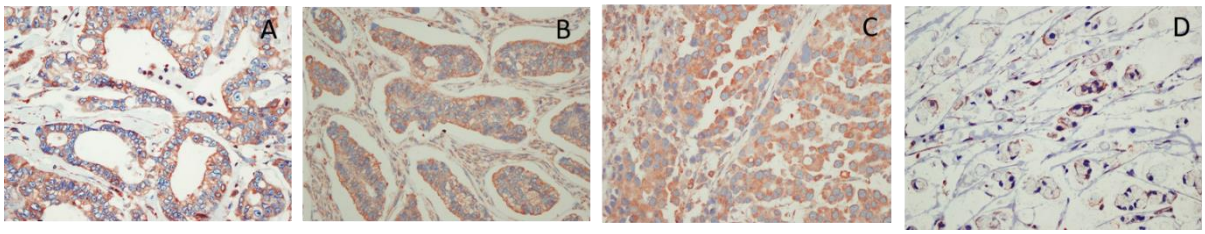

FIGURE 2: (A& B) EGFR Positivity (Membranous Staining) in Gastric Adenocarcinoma Cells C& D EGFR Positivity (Membranous and Cytoplasmic Staining) in Gastric Undifferentiated Carcinoma Cells in A (x400) EGFR Positivity (Membranous Staining) in Ring Cell Carcinoma with Gastric Stone in B (x400)

There was no correlation between EGFR expression and either age (p=0.977), time (p=0.635), the number of lymph nodes (p=0.814), or tumour size (p=0.960), as determined by the t-test. Gender (p=0.324), stage (p=0.785), microscopic type (p=0.906), lymph node metastasis (p=0.298), and tumour type (p=0.946) were found to have no correlation with EGFR expression. Fisher's test results show no correlation between EGFR expression and macroscopic type (p=0.349). The log-rank test was used to analyse the correlation between EGFR expression and survival times. It was shown that there was no correlation between EGFR expression and mortality in this study (p=0.306).

Mutagenic PCR-RFLP Results

As a result of mutagenic PCR performed to detect K-ras codon 12 genotypes, a product of 162 bp was obtained (Figure 3). Mutagenic PCR products were digested with the BstN1 restriction enzyme. After slaughter, 133 and 29 bp DNA fragments of PCR products of wild types that do not carry the mutant allele, 162, 133 and 29 bp DNA fragments of PCR products of heterozygotes carrying the mutant allele in only one allele, and PCR products of homozygotes carrying the mutant allele in both alleles. It consisted of 162 bp DNA fragments.

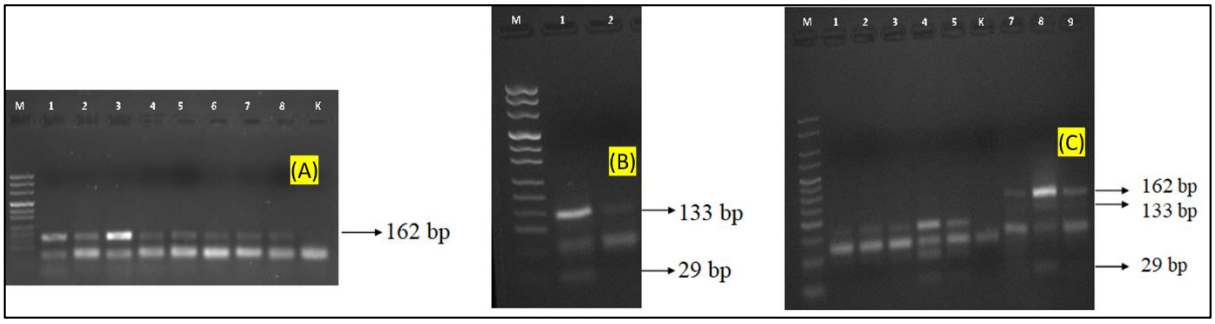

FIGURE 3: (A) 2% agarose gel view of mutagenic PCR products carrying K-ras codon 12 polymorphic regions. M: PUC8 DNA molecular weight standard; 1-8. wells: 162 bp PCR products; K: control; (B) & (C): 4% agarose gel image of K-ras codon 12 genotypes obtained by cutting mutagenic PCR products with BstN1. M in A: PUC8 DNA molecular weight standard; 1,2. wells G/G genotype (wild type) M in B: DNA molecular weight standard of 25 bp; 7-9. wells G/C genotype (heterozygous mutant). Other wells in B K-ras codon 13 genotypes (wells 1, 2, 3 C/G genotype (heterozygous mutant); wells 4,5 C/C genotype (wild type); K: control)

Allelic polymorphisms, absence of K-ras codon 12 mutation in both alleles (wild type); It can be explained by the presence of both alleles (homozygous mutant) and only one allele (heterozygous mutant) (Fig. 3B, C).

In gastric cancer patients, 44 individuals were found as wild type and 3 as heterozygous mutant. No homozygous mutation was found. The distribution frequencies of the patients' K-ras codon 12 genotypes are as shown in figure 4. In patient groups, the frequency of GG genotype was higher than both GC and CC genotypes.

Since the frequency of carrying K-ras 12 mutations in sick individuals was 6.38% (3/47), these mutations were not significant in terms of gastric cancer risk according to the Fisher test (p= 1.000). No significant correlation was found between K-ras codon 12 mutation and survival according to log-rank test (p=0.815).

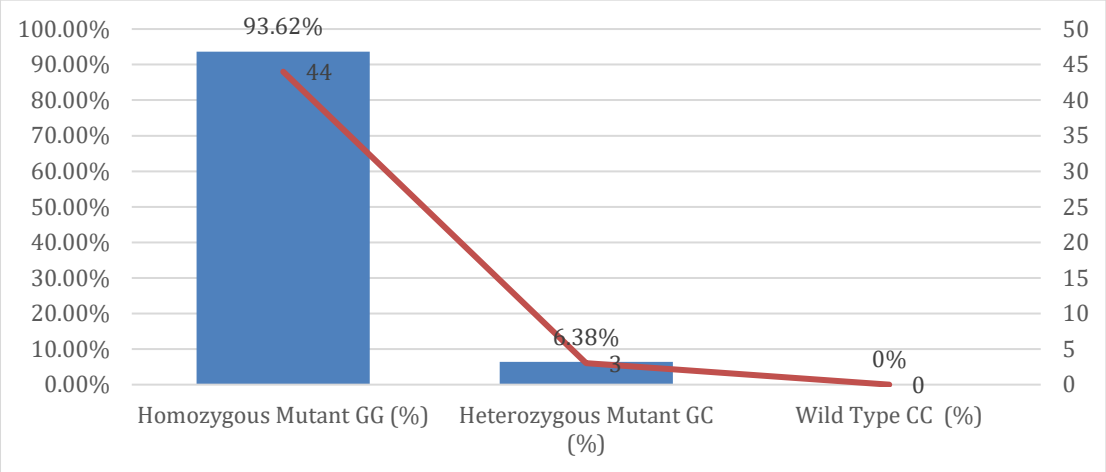

FIGURE 4: K-ras codon 12 Genotype Distributions of the Patient Group

As a result of mutagenic PCR performed to determine K-ras codon 13 genotypes, a product of 159 bp was obtained (Figure 5). Mutagenic PCR products were digested with HaeIII restriction enzyme. After slaughter, 85, 48 and 26 bp DNA fragments of PCR products of wild types without mutant allele, 85 and 74 bp DNA fragments of PCR products of heterozygotes carrying the mutant allele in only one allele, and PCR products of homozygotes carrying mutant allele in both alleles. 159 bp DNA fragments.

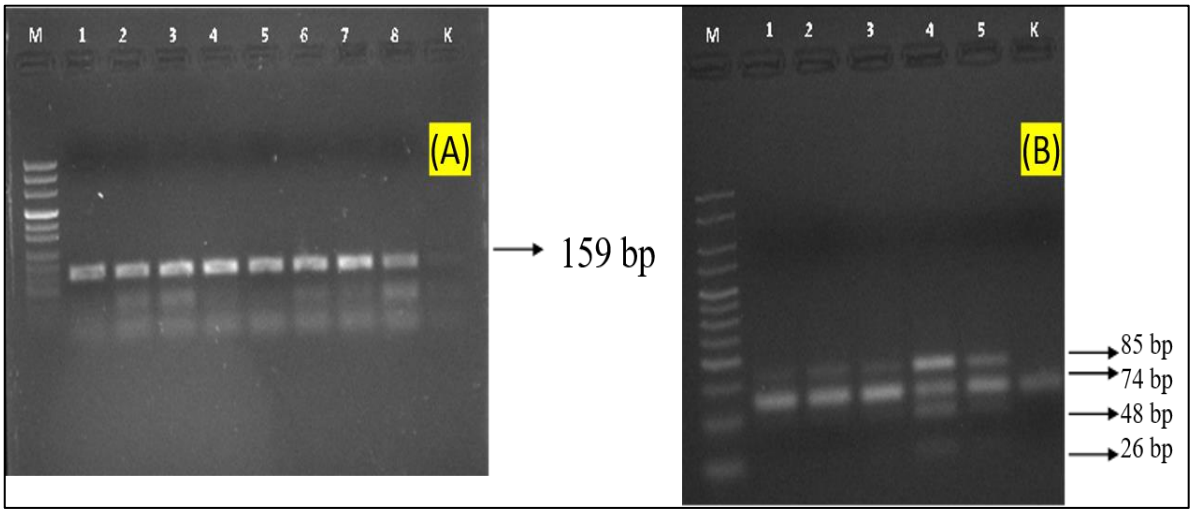

FIGURE 5: (A) 2% agarose gel view of mutagenic PCR products carrying the K-ras codon 13 polymorphic region. M: PUC8 DNA molecular weight standard; 1-8. wells: 159 bp PCR products; K: control (B) 4% agarose gel image of K-ras codon 13 genotypes obtained by cutting mutagenic PCR products with HaeIII. M: DNA molecular weight standard of 25 bp; Wells 1, 2, 3 C/G genotype (heterozygous mutant); 4.5. wells C/C genotype (wild type); K: control

Allelic polymorphisms, absence of K-ras codon 13 mutation in both alleles (wild type); It can be explained by the presence of both alleles (homozygous mutant) and only one allele (heterozygous mutant) (Fig. 5). In gastric cancer patients, 20 individuals were found as wild type and 7 as heterozygous mutant. No homozygous mutation was found. The distribution frequencies of the patients' K-ras codon 13 genotypes are as shown in figure 6. The frequency of CC genotypes in the patient groups was higher than both CG and GG genotypes. Since the frequency of carrying K-ras 13 mutations in sick individuals was 25.93% (7/20), these mutations were not significant in terms of gastric cancer risk according to the Fisher test. (p= 0.616) No significant correlation was found between K-ras codon 13 mutation and survival according to log-rank test (p=0.655).

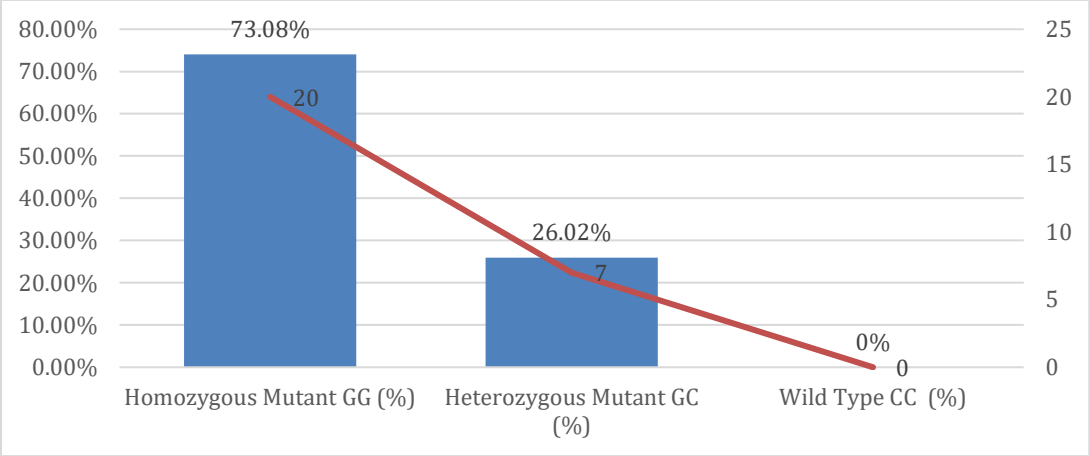

FIGURE 6: K-ras Codon 13 Genotype Distributions of the Patient Group

Comparison of immunohistochemistry and PCR-RFLP results

According to the Fisher test, no significant correlation was found between EGFR expression negative staining (-) and EGFR expression positive staining (+) and K-ras codon 12 and 13 mutation (p= 1.000, p= 0.616). Since the frequency of carrying K-ras 12 and 13 mutations in sick individuals is 6.38% and 25.93%, respectively, these mutations do not carry any significance in terms of gastric cancer risk.

DISCUSSION

Numerous environmental and genetic variables can contribute to gastric cancer. The median age of our sample is comparable to data from other countries. M/F = 1.58 Typically, 56 is when stomach cancer is discovered. Compared to western countries, where individuals typically live into their 70s, our average age is 10 years lower (4, 5). In 2008, 840 Turkish patients had an average age of 57 when stomach cancer was discovered (age range 19-85).[10]. The age of our 62 patients was on average 60.4. (age range 30-92). The disparity, in our opinion, was brought on by the tiny patient population. Unstudied differences between men and women in stomach cancer survival.

In the antrum, corpus, and cardia, respectively, 44.5 %, 41.3 %, and 7.5 % of tumours were detected in a 2008 Turkish oncology group study[10]. In our investigation, the antrum, the corpus, the cardia, and the esophagogastric junction all experienced a 50 % tumour development rate. Diffuse involvement was 9.68 %. More people have cardiac cancers. 15 % of stomach tumours in Turkey are cardiac [11]. One patient (1.61 %) in our research group recorded an increase in esophagogastric junction tumors. Gastroesophageal junction and cardiac malignancies are still common despite a significant drop in fundus and distal tumours in Western nations [12]. Despite improvements in stomach cancer treatment, antral tumours still outnumber corpus and cardia tumours in frequency.

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Stage I and II stomach tumours made up 33% of all cases, stage III tumours made up 14%, and stage IV tumours made up 40%, according to Alberts et al [13]. Patients in stage I, stage II, stage III, and stage IV made up 8.4%, 14.5%, 56.7%, and 14.5 % of the total population in 2008. [11]. Stages I, II, III, and IV patients comprised 8.6%, 41.94%, and 8.6% of the total patient population.

The majority of stomach cancers (more than 90%) are adenocarcinomas; the remainder are lymphomas and sarcomas. 48.39 % (30/62) of the sample in our study had adenocarcinoma. Undifferentiated cancer made up 25.81 % (16/62) of the cases, signet ring cell carcinoma, 8.06 % (5/62) neuroendocrine carcinoma, and mucinous carcinoma, which made up 4.45 % (4/62) of the cases, 11.29 % (7/62). The disease's diagnosis, treatment, and prevention will be made easier by SNPs in genes linked to stomach cancer. It is possible to stop progression by conducting gene mutation screenings when cancer is still difficult to detect. K-ras codon 12 mutations, in studies, enhance the spread of gastric cancer tumours [14]. Gene mutations may be an indication of cancer susceptibility and treatment response.

K-ras gene mutations, which are essential for comprehending the genetic aetiology and clinical implications of gastric cancer, are detectable by PCR and RFLP. Overexpression of EGFR can be found via immunohistochemistry.

Colon and stomach cancers have a connection to the K-ras gene polymorphism. Mutations in K-ras were found in 3% of stomach malignancies and 34% of colon tumours. Only two codon 12 and one codon 13 K-ras mutations were found in 66 gastric cancer patients. There were 1/66 mutations in K-ras codons 12 and 13. (1.5 %). 47 (28%) and 13 K-ras codon 12 mutations were found in 170 colon cancer patients (7.6 %). There are 3/170 mutations in K-ras codons 12 and 13. (1.8 %). Similar mutations were present in the majority of patients with metastatic colon and stomach cancer [15]. According to our research, 12 patients had K-ras codon 3/47 (6.38 %) and 13 patients had K-ras codon 7/27 (25.93 %). Since diet is a risk factor for stomach cancer, we believe this discrepancy may be caused by the Iraqi diet. We found no association between K-ras mutations and macroscopic types. Clinical and pathological characteristics did not correlate with K-ras codon 12 mutations. After 26 months, survival was no difference between patients with the K-ras codon 12 mutation and those who did not. Since other factors besides the stomach tumor's position may contribute to the aetiology of gastric cancer, the link between K-ras mutations in the stomach and vertical tumour localization is crucial in this study. Similar numbers of mutations in K-ras codon 12 were discovered in our study. In our investigation, we could not find any correlation between clinicopathological characteristics, survival, and K-ras codon 12 mutations. K-ras mutations in codons 12, 13, and 61 were found using PCR and dot-blot. Codon 12 (GGT, GAT 3, GTT 1) had a mutation in 4 (20%) of the 20 intestinal tumours that were examined. Sequencing of these mutations was done. No K-ras mutation was found in 11 diffuse tumours [16]. Mutations in K-ras codon 12 are more prevalent in this study than in ours. We believe that the discrepancy is due to the study's limited sample size.

K-ras codons 12, 13, 45, 69, and 80 had 33.7 % (34/101) mutations. Thirty (88.2%) of the 34 mutations were in K-ras codon 12 and one in codons 13, 45, 69, and 80. K-ras mutations are common in both Chinese and European colon cancers. Thyroid tumours have ras oncogene mutations in 20-60% of cases. We believe the study's small sample size impeded mutation identification. Survival and K-ras codon 12 had no correlation (p=0.815). K-ras codon 13-point mutation was wild type (CC) in 74.07 % (20 individuals) of 62 gastric cancer patients and heterozygous (CG) in 25.93 % (7 individuals). The mutations of 20 patients could not be found using paraffin blocks. There were no GG mutations detected. The CC genotype was more prevalent in patients than the CG and GG genotypes. Because K-ras 13 mutations are found in 25.93% (7/20) of sick persons, they do not raise the risk of gastric cancer (p= 0.616). Survival and kras codon 13 had no correlation (p=0.655).

Treatment with cetuximab prescribed for K-ras wild-type was investigated in Belgium. Immunohistochemistry for EGFR and RT-PCR for K-ras codon 12 and 13 mutation were used using paraffin tissue samples from 113 metastatic colon cancer patients. All 113 patients were found to be EGFR positive. K-ras mutations were detected in 46 (40.7%) of 113 patients [17]. Antibodies developed against EGFR Cetuximab and Panitumumab have been approved in Germany.

Cetuximab blocks the EGFR signalling pathway. In the case of mutated K-ras, EGFR blockade fails to suppress the pathway, and the tumor continues to grow and spread. Colon cancer treatment with monoclonal antibodies Sebastian Stintzing et al.[18] Researched in Germany in 2009. EGFR expression is detected by immunohistochemistry method. EGFR expression can be detected in 70% of metastatic colon cancer patients. In our study, EGFR expression in gastric cancer was found to be 77.42% (48/62) by immunohistochemistry method. It is used with PCR-based methods for the detection of K-ras codon 12 and 13 mutations. K-ras mutation is detected in 30-49% of colon cancer. K-ras mutation is found in about 40% of nonhereditary colon cancer. Cetuximab has revolutionized targeted therapies in the first-line treatment of patients with metastatic colorectal cancer. K-ras wt tumors are predictive biomarkers for Cetuximab therapy. K-ras is the first biomarker used in targeted therapies in combination with standard chemotherapy in the first-line treatment of patients with mCRC. Cetuximab treatment reduces the risk of progression in mCRC patients with K-ras wt tumors. K-ras gene analysis should be performed before determining the treatment strategy of mCRC primary care patients. For this reason, comparison studies should be conducted according to the K-ras mutation of the responses of those using these drugs. The number of patients in the study should be increased and mutation frequencies should be confirmed by sequencing. First of all, K-ras mutation screening should be performed for codons with mutations in K-ras codon 61 and later in other cancer types.

CONCLUSION

This study looked at K-RAS mutations and EGFR expression as prognostic and predictive markers in patients with gastric cancer. In the study, paraffin blocks were employed. The fact that the study's patient population's demographics typically matched that of the literature suggests that this group may be representative of all people with stomach cancer. Our study included a small number of patients, and although there was a significant correlation between EGFR expression and disease stage, age, time, number of lymph nodes, tumour diameter, gender, microscopic type, lymph node metastasis, tumour type, and survival, there was no correlation in gastric cancer. demonstrates that the pathological outcomes are not connected.

Stage, age, and microscopic type are significantly correlated with survival, indicating that these variables may be clinically meaningful survival parameters. There was no connection between K-ras codon 12 and 13 mutation, survival, or EGFR expression. In conclusion, Cetuximab (Merck-Serono) effectively suppressed proliferation in patients with positive (+) EGFR expression and wild-type alleles (C/C and G/G) for K-ras codon 12 and 13 mutations. Based on the findings of our study, we hypothesise that this medication may also be helpful in treating stomach cancer due to the high frequency of wild-type alleles in the Turkish population. The findings imply that routine use of the analysis of these mutations in the treatment of stomach cancer is possible. The mutation frequencies discovered through sequencing will be confirmed. We expand the study's patient base to continue the investigation. Additionally, we'll check for K-ras codon 61 mutations.

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