KRAS Codon 12 and 13 Mutations in Gastric Cancer in the Northeast Iran

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Background & objective: KRAS mutations are reported in many types of cancers including pancreas, lung, colon, breast, and gastric (GC). High frequency of KRAS mutation is observed in the pancreas, colon, and lung cancers; they commonly arise in codon 12 and 13 of exon 2. Due to the lack of information about the frequency of KRAS mutations in the Northeast of Iran, the current study aimed at evaluating KRAS frequency in cases with GC in this region.

Methods: A total of 120 formalin-fixed, paraffin-embedded blocks of patients with GC were assessed. The assays to detect KRAS in codon 12 and 13 were obtained through the peptide nucleic acid (PNA)-clamp.

Results: Totally 87 male and 33 female patients were analyzed in the current study. The mean age of the subjects was 55 years. The most common tumoral fragment was located on the body with 48 cases (40%) and the less frequent was related to fondues with six cases (5%). Of the 120 GC samples, 16 (13.3%) cases had codon 12 KRAS mutation, and 16.7% had codon 13 mutations. There were no significant relationships between gender, age, and KRAS mutations in the studied specimens.

Conclusion: In conclusion, the overall frequency of KRAS codon 12 and 13 mutations in GC was 30% in the current study population. Frequency of KRAS codon 12 and 13 mutations had significant correlation with tumors location. Different pathogenic mechanisms are suggested for GC according to tumor location. The current study result may be an important diagnostic tool for physicians managing atrophic gastritis.

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KEYWORDS

K-ras; Mutations; Gastric Cancer

ABSTRACT

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Introduction

Cancer is a global health challenge with high mortality; it is reported as the cause of 7.6 million deaths in 2008 (1). Gastric cancer (GC) is known as the feature common cancer worldwide, and has the second rate of mortality among cancers (2). Many patients with GC present with advanced disease and undergo treatment with preparative cytotoxic combination chemotherapy and surgery; they follow chemotherapy in the east and surgery in the west (1-3). In Iran, Northern and Northwestern regions are high risk areas for GC, but there are several low and intermediate risk regions in other geographical areas (4). Pathogenesis of gastric adenocarcinomas shows a classic example of gene-environment interaction; therefore, geographical differences of the frequency of gastric adenocarcinomas may be due to genetic factors (5). GC is a heterogeneous disease, which progresses via different
pathways of carcinogenesis; however, causes of this genetic heterogeneity are not studied thoroughly (6).

The study by Seung-Hyun (1986) was the first report on KRAS mutations in patients with GC; since then many studies investigated KRAS mutations status in the same field. More than 80% of studies were conducted on Asian patients with GC. The largest Western study included 82 patients with GC whereas the largest Asian study from Korea included 319 cases (1,2).

GCs are classified according to tumor side and pathological characteristics of lesions. Laurens classification describes GC in four levels including differentiated, diffuse-type, infiltrated, and intestinal-type carcinomas, characterized by cohesive cells (5). K-RAS, a small G-protein belongs to RAS family and epidermal growth factor receptor (EGFR) downstream, is a necessary component of EGFR signaling cascade (6). Unusual activation of EGFR-RAS-RAF-MAP kinase pathway is observed in GC; in this cascade, activating mutations of EGFR gene induces ligand-independent activation of receptor, which leads to over activation of KRAS. Its downstream effectors play a significant role in EGFR-RAS-RAF-MAP kinase pathway is observed in GC; in this cascade, activating mutations of EGFR gene induces ligand-independent activation of receptor, which leads to over activation of KRAS. Its downstream effectors play a significant role in EGFR-RAS-RAF-MAP kinase pathway. KRAS mutations alter gene conformation and induce KRAS protein activation. In general, KRAS mutations are contradictory to EGFR mutations; they can activate EGFR-RAS-RAF-MAP kinase cascades independent of EGFR mutation. Therefore, KRAS proteins appear to be resistant to EGFR inhibitors. KRAS mutations are known as a negative predictor, it is contended whether EGFR inhibitors are useful for GC, because KRAS mutation status depends on EGFR mutation status (7).

KRAS mutations are reported in many types of cancers including pancreas, lung, colon, breast, and GCs (6,7). High frequency of KRAS mutation was observed in pancreas, colon, and lung cancers; they commonly arise in codons 12 and 13 of exon 2 (7). It was observed that DNA changes can distinguish tumor subtypes among histologically identified tumors (8). Peptide nucleic acid (PNA) is a synthetic nucleic acid, which binds to its complementary RNA or DNA sequences; PNAs have higher sensitivity, speed, simplicity, and lower cost(3). Due to lack of information about the frequency of KRAS mutations in the Northeast Iran, the current study aimed at evaluating it in GC cases.

**Material and Method**

The current cross sectional study was conducted in Ghaem Medical Center affiliated to Mashhad University of Medical Sciences, Mashhad, Iran in 2015 on 120 formalin-fixed, paraffin-embedded blocks of patients with GC. Hematoxylin and eosin (H&E)-stained sections of each tissue sample were examined by two expert pathologists to confirm histology. The selected blocks contained highest density of primary adenocarcinoma. The selected area contained more than 30% tumor cells in tissue and was marked on the slide to facilitate macro dissection. Up to 5-10-µm sections were cut and the marked areas of interest were dissected using a sterile scalpel blade. Genomic DNA was extracted according to QIAMP DNA micro kit protocol (Qiagen, Hilden, Germany).

The KRAS mutations in codon 12 and 13 were assessed by the PNA-clamp (PANAGENE, Inc., Daejeon, Korea). PNA primers and probes were associated with the commercial kit. All reactions were done in 20 µL volumes and SYBR Green was used; DNA was also used as template. The primers and PNA probes were set. PCR cycling conditions were as follows: five minutes hold at 94°C followed by 40 cycles of 94°C for 30 seconds, 63°C for 30 seconds and 72°C for 30 seconds. In this assay DNA primer and PNA probes were used together in the clamping reaction. The PNA probe sequence is complementary to wild-type DNA, suppresses amplification of wild-type targets; it enhances preferential amplification of mutant sequences by inhibiting DNA primers competitively. They bind to wild-type DNA, and positive signals are detected by intercalation of SYBR Green fluorescent dye. Efficiency of performed PCR was determined by measuring threshold cycle (CT) value. The delta CT (delta CT) value was calculated according to the following formula

\[
\text{[standard CT]} - \text{[sample CT]} = \text{delta CT}
\]

The sample and standard CT values were from the
tested and clamping control samples. The cut off delta CT was 2 for \( \text{KRAS} \) mutations.

**Statistical analysis**

SPSS version 16 was employed for statistical analysis. Chi-square and Fisher exact tests were applied. P value <0.05 was considered significant.

**Results**

The current study evaluated 120 patients with GC in order to identify \( \text{KRAS} \) mutations (codons 12 and 13); 87 patients were male and the rest of them female. Mean age of the patients was 55 years. The most common tumoral location was related to body with 48 cases (40%) and the less frequent was related to fondues with six cases (5%). Of the 120 GC samples, 16(13.3%) patients had codon 12 \( \text{KRAS} \) gene mutation and 16.7% had codon 13 mutation. There were no significant relationships between gender, age, and \( \text{KRAS} \) mutations.

The relationship of \( \text{KRAS} \) mutations with other clinical, pathological, and molecular parameters are summarized in Table 1.

| Table 1. Relationship of KRAS Mutations With Clinical, Pathological, and Molecular Parameters of Gastric Adenocarcinoma Cases |
|---------------------------------------------------------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Gender**                                                   | **Frequency of KRAS Codon 12 Mutation** | **Percentage of KRAS Codon 12 Mutation** | **P-value** | **Frequency of KRAS Codon 13 Mutation** | **Percentage of KRAS Codon 13 Mutation** | **P-value** |
| Male                                                        | (87)10                                  | 11.49                                      | 0.336      | (87) 14                             | 16                                           | 0.784                                       |
| Female                                                      | (33)6                                   | 18                                         |            | (33) 6                               | 18                                           |                                            |
| **Tumor Site**                                               | **Frequency of KRAS Codon 12 Mutation** | **Percentage of KRAS Codon 12 Mutation** | **P-value** | **Frequency of KRAS Codon 13 Mutation** | **Percentage of KRAS Codon 13 Mutation** | **P-value** |
| Cardia                                                      | (26) 4                                  | 15.38                                      | 0.250      | (26) 4                               | 15.3                                         | 0.015                                       |
| Fundus                                                      | (6) 2                                   | 33                                         |            | (6) 4                                | 66                                           |                                            |
| Body                                                        | (48) 8                                  | 16.6                                       |            | (48) 8                               | 16                                           |                                            |
| Antrum                                                      | (32) 2                                  | 6.2                                        |            | (32) 4                               | 12.5                                         |                                            |
| Pilorum                                                     | (8) 0                                   | 0                                          |            | (8) 0                                | 0                                            |                                            |
| **Tumor Type**                                               | **Frequency of KRAS Codon 12 Mutation** | **Percentage of KRAS Codon 12 Mutation** | **P-value** | **Frequency of KRAS Codon 13 Mutation** | **Percentage of KRAS Codon 13 Mutation** | **P-value** |
| Intestine                                                   | (90) 15                                 | 16.7                                       | 0.116      | (90) 14                              | 15                                           | 0.572                                       |
| Diffuse                                                     | (30) 1                                  | 3.3                                        |            | (30) 6                               | 20                                           |                                            |
| **Tumor Differentiation**                                   | **Frequency of KRAS Codon 12 Mutation** | **Percentage of KRAS Codon 12 Mutation** | **P-value** | **Frequency of KRAS Codon 13 Mutation** | **Percentage of KRAS Codon 13 Mutation** | **P-value** |
| Well                                                        | (42) 3                                  | 7                                          | 0.343      | (42) 6                               | 14                                           | 0.603                                       |
| Moderate                                                    | (48) 8                                  | 16                                         |            | (48) 10                              | 20                                           |                                            |
| Poor                                                        | (30) 5                                  | 16                                         |            | (30) 4                               | 13                                           |                                            |
| **Tumor T**                                                 | **Frequency of KRAS Codon 12 Mutation** | **Percentage of KRAS Codon 12 Mutation** | **P-value** | **Frequency of KRAS Codon 13 Mutation** | **Percentage of KRAS Codon 13 Mutation** | **P-value** |
| T1                                                          | (8) 4                                   | 50                                         | 0.011      | (8) 4                                | 50                                           | 0.016                                       |
| T2                                                          | (22) 4                                  | 18                                         |            | (22) 6                               | 27.2                                         |                                            |
| T3                                                          | (69) 6                                  | 8.7                                        |            | (69) 8                               | 11.5                                         |                                            |
| T4                                                          | (21) 2                                  | 9.52                                       |            | (21) 2                               | 9.5                                          |                                            |
| **Tumor N**                                                 | **Frequency of KRAS Codon 12 Mutation** | **Percentage of KRAS Codon 12 Mutation** | **P-value** | **Frequency of KRAS Codon 13 Mutation** | **Percentage of KRAS Codon 13 Mutation** | **P-value** |
| N0                                                          | (26) 6                                  | 23                                         | 0.064      | (26) 8                               | 30                                           | 0.115                                       |
| N1                                                          | (44) 7                                  | 15.9                                       |            | (44) 6                               | 13.6                                         |                                            |
| N2                                                          | (41) 1                                  | 2.4                                        |            | (41) 6                               | 14.6                                         |                                            |
| N3                                                          | (9) 2                                   | 22                                         |            | (9) 0                                | 0                                            |                                            |
Discussion

The current study aimed at determining the frequency of KRAS codon 12 and 13 point mutations in stomach cancer in Iranian population and comparing the results with general population. KRAS, an oncogene located on chromosome 12p12.1 plays a significant role in downstream signaling of EGFR pathway. Mutations in KRAS gene induce uncontrolled activation of RAS protein \((3)\). Ras protein transforms signals from EGFR to mitogen activated protein kinases (MAPKs). Ras protein is located in the inner region of cell membrane and controls cell growth proliferation and motility as well as metastasis and angiogenesis. The current study employed a PNA clamp-PCR method.

By PNA clamp-PCR method, 16(13.3%) KRAS codon 12 and 20(16.7%) KRAS codon 13 point mutations were observed among the 120 stomach cancer samples. In the current study, 30% of all GCs showed KRAS codon 12 and 13 mutations; the current study data were similar to those of Polane et al., which reported 30.9% as frequency of KRAS mutations in patients with GC (9).

Grieken et al., in UK reported KRAS mutations in 4.2% of cases with GC (2). In a series of studies, the frequency of KRAS gene mutations were 11.8%, 21%, and 11.4% in patients with GC (10), (11), (12), (13). In contrast to the current study results, Motsova et al., (2013) detected KRAS mutations in 1% of similar patients. Some of the reasons for disagreements between the findings of the study by Motsova et al., and those of others attribute to sample sizes, different methodologies, and different analysis methods of the mentioned gene mutation.

Of the 120 studied gastric adenocarcinomas, 13% had KRAS mutation codon 12 and 16% codon 13 (Table 1). This result was consistent with those of other recent studies of GC which revealed low incidence of KRAS mutations (7%-20%) (10). There was a significant correlation between KRAS mutation codons and sites; 33% of KRAS mutation codon 12 and 66% of codon 13 arose from gastric fundus \((P=0.015)\). Zhao et al., showed that all eight KRAS mutant gastric tumors arose from gastric antrum \((P=0.002)\) (8), but three were from gastric body. Most of the current study KRAS mutated cases were moderately differentiated tumors, while most of KRAS mutant cases reported by Zhao et al., were well differentiated tumors (14); 50% of KRAS mutations codon 12 and 13 were T1 tumor \((P=0.011, P=0.016)\), but other studies did not report significant correlations between KRAS mutations and TNM stage (12). In the current study, the highest frequency of KRAS mutations was observed in stage I (28.5%), this result was similar to that of the recent study by Zhao et al. (21%)(14). Almost all of the current study KRAS mutations were diffuse type, which was in contrast with those of other studies. It is mentioned that KRAS mutations are detectable in intestinal type and it is not a feature of diffuse type carcinoma (15).

Conclusion

In conclusion, the overall frequency of KRAS codon 12 and 13 mutations in GC in the current study population was 30%. Frequency of KRAS codon 12 and 13 mutations showed significant correlation with tumors.
KRAS Codon 12 and 13 Mutations in ... location. This information may be an important diagnostic tool for physicians managing patients with atrophic gastritis.

**Conflict of Interests**
Authors declared no conflict of interests.

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