Research article

Genetic mutation rs972283 of the *KLF14* gene and the incidence of gastric cancer

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ABSTRACT

Introduction and Aim: Genetic factors and family gene clustering constitute an important ratio for gastric cancer. Kruppel Like Factor 14 (*KLF14*) gene has a carcinogenic role and a clear role in metabolic diseases, but how this gene regulates these metabolic traits is still obscure. Previous studies proposed that the accumulation of single nucleotide polymorphisms (SNPs) in *KLF14* may be associated with gastric cancer. The current study aimed to investigate whether single nucleotide polymorphisms (SNP) rs972283 in *KLF14* is associated with an increased risk of gastric cancer in the Iraqi population.

Materials and Methods*:* The SNP was genotyped using tetra primer ARMS-PCR in 101 (79 men and 22 women) gastric cancer patients who did not receive chemotherapy, and 80 healthy controls (53 men and 27 women). All patient samples were taken from the Baghdad Hospital of Gastroenterology and Hepatology laboratories. Patient records included age, sex, histological type, and *H. pylori* infection status

Results: The KLF14 rs972283 genotype was significantly different between the gastric cancer and control groups. The heterozygous AG genotype and A mutant allele were significantly higher in gastric cancer patients compared to controls (56.4% vs 38.7%, $p<0.01$ and 61% vs 40.6%, $p<0.01$, respectively). In contrast, the GG wildtype genotype and G wildtype allele were significantly higher in controls (40% vs 11%, $p<0.01$ and 59.4% vs 39%, $p<0.01$, respectively). The AA homozygous mutant genotype also showed a weak correlation with increased gastric cancer risk. These results indicate the A allele is a risk factor while the G allele has a protective effect for gastric cancer.

Conclusion: the KLF14 polymorphism rs972283 exhibits a significant association with gastric cancer risk in our Iraqi cohort. The SNP may serve as a useful prognostic marker, pending validation in larger studies.

Keywords: Gastric Cancer; single nucleotide polymorphism; rs972283; *KLF14* gene; Tetra Primer; ARMS-PCR.

INTRODUCTION

Gastric cancer is the third most deadly cancer in India and the fourth most common carcinogenesis globally, with 70% of the cases reported in developing countries and an estimated 8–30% of cases having a positive family history for this cancer (1-3). Gastric cancer is a heterogeneous disease characterized by multiple molecular genetic steps which are affected by various genetic and environmental factors (4,5). According to the Iraqi Cancer Registry 2011 and 2018, gastric cancer in Iraq is ranked tenth among the top ten cancers, with a 2.1/100,000 population incidence rate that is more prevalent in males than females (6).

Gastric cancer is associated with many mutations. Increased expression and activity of PARP1 enzyme has been significantly correlated with gastric cancer. Similarly, a strong association between inflammatory cytokines has been found in patients with gastric cancer (7 *KLF14* regulates cell signaling pathways, cell differentiation, proliferation, and immune system and has a role in tumorigenesis. Oxidative adaptation is induced by the *KLF14* gene in patients with prostate cancer, where the heme-oxygenase-1 signaling is modulated, leading to cancerous growth (8). There is an inverse relationship between *KLF14* expression and *PIK4* expression for both colon cancer and breast cancer. Several studies have indicated that *KLF14* gene has a regulatory role in breast, cervical, colorectal, placenta cancers (9). It was discovered that the *KLF14* gene plays a negative regulatory role in gastric cancer, resulting in chromosomal instability.

Recent studies have linked the *KLF14* gene to cancer via boosting the activity of basal carcinoma cells, suggesting that this gene may serve as a diagnostic marker and a therapeutic target for cancer (10). Considering the carcinogenic susceptibility of the *KLF14* gene, the aim of this study was to molecularly detect *KLF14* gene in Iraqi patients with gastric cancer using tetra primer technique, and to determine the use of *KLF14* as a prognostic marker and target for gastric cancer treatment as well as the association with clinical signs such as sex and age.

MATERIALS AND METHODS

Samples

In this study, 80 samples were collected from healthy volunteers (53 males and 27 females) and 101 from

patients (79 males and 22 females) with gastric cancer who did not receive chemotherapy. All patient samples were taken from the laboratories of the Hospital of Gastroenterology and Hepatology in Baghdad, after obtaining official approval from the hospital administration and also from patients. Demographic data of each patient such as age, sex, histopathology type, infection with *H. pylori* etc., were obtained from patient records.

DNA extraction

Genomic DNA was extracted from all samples by using DNA Tissue kit (Qiagen, German) according to the manufacturer's instructions. The PCR reaction was carried out in a total volume of 20 µL, containing 6 µl of template DNA, 0.8 µl of forward inner primer (G allele), 0.8 µl of reverse inner primer (A allele), 1.2 µl for reverse outer primer, 1.2 µl for forward outer primer, and 10µl green master mix (Promega, USA). The cycling conditions were as follow: initial denaturation at 94˚C for 3min, followed by denaturation at 94˚C for 1 min with 30 cycles, annealing at 60˚C for 1 min, extension at 72˚C for 1 min, and more extension at 72˚C for 10 min at the30 cycles, DNA hold at 4˚C (11). Specific KLF14 gene primers used in the Tetra Primer ARMS-PCR are given in Table 1.

The PCR amplified DNA products were separated by electrophoresis in 1% agarose gel and stained with 0.4 µl ethidium bromide. The bands were visualized and photographed under UV transillumination. Gel image of representative samples showing the expected band sizes are shown in Fig. 1.

Table 1: *KLF14* Primers used in tetra Primer ARMS PCR

SNP	Primer sequence (5' to 3')	Base pair
rs972283 g.130782095 (A/G)	Forward outer primer F1: GTCATAGGTCAAACAGCTAGATATTGGGT	437 bp
	Reverse outer primer R1: TCTACAGGACCAACTCAAATTATGAGGT	
	Forward inner primer (G allele) F2: TCATTGTATACTTGGAAAAAATCCTACATG F2 and R1	G allele 221 bp
	Reverse inner primer (A allele) R2: TATGTAAAAATAAGTATGCGCCATGCCT F1 and R2	A allele 274 bp

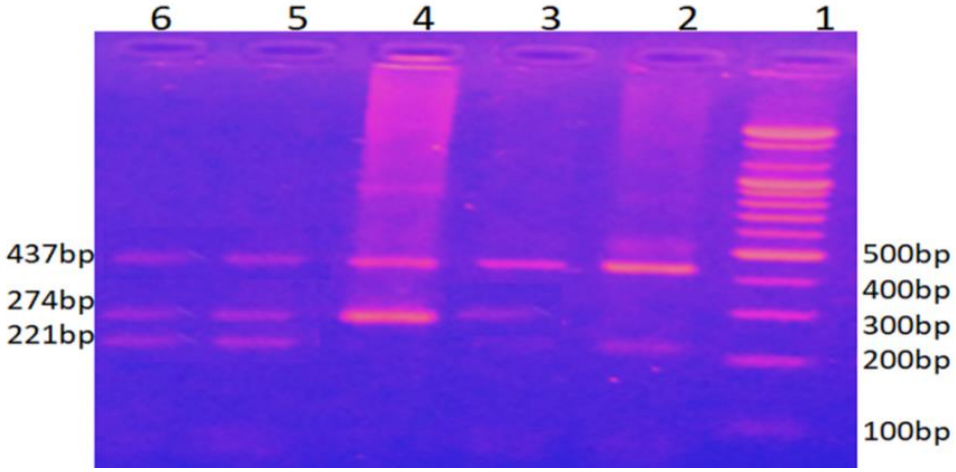

Fig. 1: Tetra Primer ARMS-PCR amplification of DNA samples. Lane 1: DNA ladder (100 bp) , Lane 2: GG homozygous genotype two band 437 bp and 221 bp; lanes 3 and 4: AA homozygous genotype two band 437 bp and 274 bp ; lanes 5 and 6: AG heterozygous genotype three band 437 bp, 274bp and 221 bp .

GTCATAGGTCAAACAGCTAGATATTGGGTTATCTGCCAGGTAAAAGAAGTATTCT
GTGAAAGACACACAGTAAATACCCATGTCAGGCTCCTCCCCATTTCTCATCAGTGCA
GGGTCTCTAGCTGCTCTGCTACTGGAATCCCAGTTTAGCTGGAGTCTCTCGGAACAG
TCCGTTAATATGGTTGATTGCATTGGTTGATTTTCAGCTATTGAACCATCATTGTAT
ACTTGGAAAAAATCCTACCT[G/A]GTCATGGCGCATACTTATTTTTACATATTAC
TAAACTGTATTTGCTAATATTTTGTTTAAAATTTTGCGTCTGTATTCGTGACAGATCT
TCCTCCCTCCCTCCCTCCCTCCCTCCTTTCCTCTTTGTATTTTCTTGGTCAAGTTTTGAT
AATAGTGAAACTGACCTCATAATTTGAGTTGGTCCTGTAGA

Fig. 2: The *KLF14* gene segment *was* amplified in the current study. The sequence pertains to Homo sapiens chromosome 7, GRCh38.p13 Primary Assembly NCBI Reference sequence (NC_000007.14) region: c130782285-130781849.

Amplification of *KLF14* gene

Detect the g.130782095 A>G (rs972283) SNP *KLF14*, GG genotype of 437 bp from binding outer primers F1 (GTC…) and R1(ACC…) amplify the outer region, AG genotype G allele of 221bp from primers F2 (TCA…) and R1, and AA genotype A allele of band 274 bp from primers F1 and R2 (TAT…) (Fig. 2).

Statistical analysis

The Statistical Analysis System (SAS, 2012) program was used in statistical analysis (12). The Odds ratio calculated was subjected to Chi-square test, to find the significant differences between percentages (0.01 probability).

RESULTS

The research encompassed a cohort of 101 individuals diagnosed with stomach cancer, as well as a control group consisting of 80 individuals who were deemed healthy. The proportion of stomach cancer patients was found to be significantly greater among males in comparison to females (88% vs 12%, p<0.01). There was a statistically significant difference in the incidence of gastric cancer between patients aged 50 years or older and those younger than 50 years (86.2% vs 13.8%, p<0.01). The prevalence of the diffuse histological subtype was found to be substantially higher compared to the intestinal subtype (62.4% vs 37.6%, p<0.01) (Table 2).

Table 2: Distribution of patients according to the age and gender groups

Factors	Patients (n=101)	P-value
Male	89 (88%)	0.0001
Female	12 (12%)	
Age <50	14 (13.8%)	0.0001
Age ≥ 50	87 (86.2%)	
Intestinal	38 (37.6%)	0.0129
Diffuse	63 (62.4)	

* (P≤0.05), ** (P≤0.01).

Distribution of diffuse and intestinal types of gastric cancer

Table 3 displays a statistically significant difference (P≤0.01) in the diffuse type between females (75%) and males (60.7%). Our results also revealed the prevalence of diffuse type gastric cancer to be highly prevalent among individuals aged below 50 years (Table3). Although the prevalence of intestinal type of gastric cancer was found to be higher in patients aged ≥50 years, no significant difference was seen for the occurrence of diffuse or intestinal type of gastric cancer in this age group (Table 3).

Table 3: Distribution of diffuse and intestinal type of gastric cancer according to the gender and age groups

Factors	Diffuse	Intestinal	P-value	Chi-square
Male (n=89)	54 (60.7%)	35 (39.3%)	0.0440	4.056*
Female (n=12)	9 (75%)	3 (25%)	0.0833	3.00 NS
P-value	0.0001 **	0.0001 **		
Age <50 (n=14)	11 (78.6%)	3 (21.4%)	0.0325	4.571*
Age ≥50 (n=87)	51 (58.6%)	36 (41.4%)	0.1078	2.586 NS
P-value	0.0001 **	0.0001 **		

* (P≤0.05), ** (P≤0.01), NS: Non-Significant.

Table 4: Genotype and allele frequency of *KLF14* gene

Genotype	Patients (n=101)	Controls (n=80)	O.R. (C.I.)	Chi-square
GG Wild type	11 (11%)	32 (40%)	Reference	10.256 **
AG Mutant Heterozygous	57 (56.4%)	31 (38.7%)	1.272 (0.79-2.07)	7.681 **
AA Mutant Homozygous	33 (32.6%)	17 (21.3%)	0.892 (0.5-1.62)	5.120 *
Allele frequency				
G	79 (39%)	95 (59.4%)	---	1.471 NS
A	123 (61%)	65 (40.6%)	---	17.893 **

* (P≤0.05), ** (P≤0.01).

Allele frequency of KLF14 gene

A notable disparity in genotyping patterns was observed between the control and patient cohorts (Table 4). In the group of patients, there was a notable increase in the prevalence of the AG genotype (56.4%) compared to the control group (38.7%) (OR=1.272; chi-square = 7.681; P≤0.01).

Additionally, the mutant A allele was found to be more prevalent in gastric cancer patients (61%) compared to the control group (40.6%). These findings suggest a significant association between the AG mutant genotype and the mutant A allele with gastric cancer. The frequency of the AA genotype was found to be 32.6% in the patient group, whereas it was 21.3% in the control group. This observation suggests a statistically significant but weak connection, indicating that the AA genotype does not confer a significant pathogenic effect (OR=0.892; chi-square = 5.120; (P≤0.05). This suggests that individuals with the AA genotype exhibit a decreased susceptibility to the development of gastric cancer. The prevalence of

the GG wild genotype was found to be substantially greater in the control group (40%) compared to gastric patients (11%). Similarly, the G wild allele was more prevalent in the control group (59.4%) compared to gastric patients (39%). This difference in prevalence was statistically significant, as indicated by a chi-square value of 10.256 and a p-value of less than or equal to 0.01. These findings are summarized in Table 4. The AG genotype is indicative of a risk genotype, while the A allele is associated with a risk allele that is linked to stomach cancer. Conversely, the GG genotype and G allele are indicative of a protective genotype and a protective allele, respectively, for the control group.

DISCUSSION

Greatest cases of stomach cancers are gastric carcinomas, which can be classified into a number of subtypes (13). The present study observed a significant predominance of male patients (88%) compared to female patients (12%) in the cohort of individuals diagnosed with stomach cancer ($p<0.01$). This finding contradicts the results reported by Zhen *et al.,* where they observed a higher prevalence of elevated *KLF14* expression in females (39 out of 185) compared to males (21 out of 185) among colorectal cancer patients (14). In Iraq, a previous study suggests that depending on gender, male patients with gastric cancer were significantly higher in comparison with female patients, they were (69.5%), and (30.5%) respectively (15). There is a significant difference between males and females with gastric cancer, where the incidence of infection increases in females when the age <44, while it increases for males when the age is ≥45 (16). No significant differences were identified between males and females with gastric cancer in terms of mortality risk, and equal diagnosis rates were established for both sexes (17). In the present investigation, it was shown that patients aged 50 years or over exhibited a greater risk compared to those younger than 50 years (86.2% vs 13.8%, $p<0.01$). Additionally, the diffuse subtype was found to be more prevalent than the intestinal subtype (62.4% vs 37.6%, $p<0.01$). This is consistent with a study that indicated that there is a significant difference in the ratio of ages >50 than ages ≤ 50 (6, 18). No significant difference was observed in the incidence of gastric cancer between patients aged ≤ 60 and patients aged >60 (19). This study showed that the diffuse type was a high significant ($P\leq0.01$) difference for females (75%) versus males (60.7%). Male patients (531) with gastric cancer were higher than females (277); in addition, 41.6% (221/531) of males were intestinal type and 58.4% (310/531) were diffuse type according to Lauren classification (20). Prior studies have indicated a gender disparity to exist for the two types of gastric cancer. A study by Kalff *et al.,* (21) reported that the prevalence of intestinal type of gastric cancer was higher in males compared to females, while the prevalence of the diffuse type was highly prevalent in females in comparison to males. Sato *et al.,* showed 70% of patients with stomach cancer were of the intestinal type, where the intestinal type was more prevalent in males, while the metastatic type was more prevalent in females (16). These findings support that the female sex hormone might be protective against intestinal-type gastric cancer (22).

Age has been also associated with the onset and type of gastric cancer developed. In this study, the onset of diffuse type of gastric cancer was statistically significant at age <50 years, while the intestinal type was significant at the age ≥ 50. This is consistent with other studies of patients with gastric cancer where the diffuse type was statistically significant in patients aged <50 (23). The poor carcinoma would agree to the diffuse type while the tubular and papillary carcinomas would agree to the intestinal type (24,25).

Regarding the genotype, this study revealed a higher prevalence of the AG genotype (56.4% vs 38.7% controls, $p<0.01$) and A allele (61% vs 40.6%, $p<0.01$) among gastric cancer patients, indicating an association with an elevated risk of developing cancer. The prevalence of the GG genotype and G allele was significantly lower in patients (11% and 39%) compared to controls (40% and 59.4%, $p<0.01$), indicating a potential protective impact. The presence of AG and A alleles are considered as risk factors, but the presence of GG and G alleles may confer a protective effect against the development of gastric cancer. KLF14 gene polymorphisms may serve as gastric cancer biomarkers or therapeutic targets. The *KLF14* gene has been associated with different genomic variants that are highly correlative with gastric cancer development and as a predictive marker for colon cancer (26). Previous studies reported the association of autosomal genetic disorders in the *KLF14* gene and gastric cancer. Thus, mentioned that rs157935 SNP in the *KLF14* gene increased the risk of developing basal cell carcinoma (27). The *KLF14* sequence is highly variable; this variability may be due to the transcript's monoallelic expression, which allows for the accumulation of mutations on the silenced allele (28). The *KLF14* rs972283 A>G gene was more associated with PCOS, and the AA genotype was strongly present compared to the AG and GG genotypes where the A allele was the risk factor (26). The *KLF14* rs972283 A>G are significantly associated with gene expression regulation in adipose tissues. There was a significant association between the MMP-7-181 (A>G) polymorphism and the risk of gastric cancer (29). The *KlF14* is highly up-regulated in multiple carcinomas associated with colon, breast cancer and peptic ulcer. The negative regulatory role of the *KLF14* gene leads to genetic instability associated with gastric, colon, and breast cancer (27).

CONCLUSION

This study demonstrated that the *KLF14* gene SNP rs972283to be significantly correlated to gastric cancer. This finding suggests that the variability within this SNP of the *KLF14* gene could potentially be used as a viable approach for monitoring gastric cancer instances, pending further investigations with larger and more diverse population samples.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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