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Research Article

CDH1 gene mutations and its association with diffuse gastric cancer

Abbas Moridnia[1], Mohammad Amin Tabatabaiefar[1], Mehrdad Zeinalian[1], Mohammad Minakari[2] and Majid Kheirollahi*[1]

[1]Pediatric Inherited Diseases Research Center,
Research Institute for Primordial Prevention of Non-communicable disease and Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[2]Internal medicine department, School of Medicine,
Isfahan University of Medical Sciences, Isfahan, Iran
*Corresponding Author: MajidKheirollahi, Ph.D.,
Associate Professor of Pediatric Inherited Diseases Research Center,
Research Institute for Primordial Prevention of Non-communicable disease and
Department of Genetics and Molecular Biology, School of Medicine,
Isfahan University of Medical Sciences, Isfahan, Iran.
P.O.Box: 81746-73461, Tel: +98(31)37922486 & Fax: +98(31)36688597,
Email: mkheirollahi@med.mui.ac.ir
Contact information: AbbasMoridnia, Ph.D, Email: a_moridnia@med.mui.ac.ir
Mohammad Amin Tabatabaeefar, Ph.D, Assistant Professor,
Email: tabatabaiefar@med.mui.ac.ir
MehrdadZeinalian, Ph.D, Assistant Professor,
Email: zeinalianmehrdad@gmail.com
Mohammad Minakari, M.D, Associate Professor, minakari@med.mui.ac.ir

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ABSTRACT

Background: Gastric cancer (GC) is one of the common cancers in world and second cause of cancer death. *CDH1*gene mutations are the important cause of diffuse gastric cancer. E-cadherin protein is coded by *CDH1* gene. In present study, we assess the nucleotide changes in *CDH1* gene among diffuse gastric cancer patients.
Materials and methods: We assessed 45 patients of gastric cancer (17 hereditary and 28 sporadic) that recognized according to the histopathological criteria and family history. DNA extracted from formalin fixed paraffin-embedded tissues and blood samples. The entirecoding region of *CDH1* gene was amplified by polymerase chain reaction.
Results: Totally, 45 patients including 7 males and 10 females in hereditary diffuse gastric cancer (DGC) and 8 female and 20 male in sporadic DGC were assessed. In hereditary DGC cases in 17.6% patients tumors were recognized in early TNM stage (I, II) versus late stages (III, IV) in 82.4%. In sporadic DGC cases 21.4% were stage (I, II) and 67.9% stages (III, IV) and 10.7% unknown. Overall, 16 variants (7 exonic and 9 intronic) was detected in HDGC patients. Contrary,sixteen variants (3 exonic and 13 intronic) were found in *CDH1* gene.
Conclusion: Totally, 64.7% of hereditary and 14.2% of sporadic samples was exhibited *CDH1*exonic variants. Our results reveal that should be considered even one cases of diffuse gastric cancer with positive family history that prominence important regarding genetic counselling.

Keywords: *CDH1* gene, Diffuse gastric cancer, Mutation, Hereditary, Sporadic

INTRODUCTION

Gastric cancer is the fourth most common cancer worldwide with 952.000 new cases and 723.000 deaths in 2012 and is the second leading cause of death(1).In Iran, gastric cancer is the second leading cause of death from cancer and the second cancer in men and fourth in women with 14% and 7% prevalence respectively(2). Most cases of stomach cancer are sporadic and familial aggregation established almost 10% and hereditary pattern have been showed in 1-3% of cases (3, 4).Thebased on histopathological classification of Lauren, gastric cancer divided into two forms include intestinal and diffuse type(5).Hereditary form of diffuse gastric cancer (HDGC) is an autosomal dominant form of diffuse gastric cancer (DGC) with aggressive, poor prognosis and high penetrance that led to thickening of the stomach wall (*Linitisplastica*) without forming a mass. Signet ring cell carcinoma (SRCC) is the most common histopathological form of stomach cancer. The *CDH1* gene mutations are the most common cause of hereditary and sporadic form of DGC(6).

The *CDH1* gene mutations in gastric cancer have inversely proportion with background of gastric cancer incidence. Therefore, in countries with a low incidence of gastric cancer, such as North America, UK and Canada incidence of *CDH1*gene mutationsis 51.6% and in the countries with moderate incidence such as Germany is 25% and 22.2% in high incidence countries such as Italy and Portugal(7). So far, germline mutations in candidate genes include *CTNNA1*, *BRCA2*, *STK11*, *SDHB*, *PRSS1*, *ATM*, *MSR1* and *PALB2* have been reported in patients with HDGC(8).In addition, several somatic mutations in the genes such as *LMTK3*, *RHOA*, *PIK3CA*, *MED1*, *ARID1A* and *MCTP22* have also been reported(9).More than 80% *CDH1* gene mutation carriers are at risk of developing gastric cancer up to 80 years. In addition, 60% risk of developing lobular breast cancer (LBC) in *CDH1* mutations carrier women are exist(10).The *CDH1* gene is located on long arm of chromosome sixteen and encompasses 16 exons that encoding for E-cadherin protein(11).E-cadherin is a glycoprotein and consisted of three domain include extracellular, transmembrane and cytoplasmic. E-cadherin has a vital role in cell-cell adhesion and tumor suppressing(12).Defecting in E-cadherin protein can result in tumor invasion and cancer development in *CDH1* mutations cancer cells(13).*CDH1* gene promoter hypermethylation is the most common mechanism that poses as a second hit in the DGC (7). The majority of germline mutations have been identified so far are single nucleotide substitution that lead to non-synonymous, deletions or frameshift mutations(14).

Aproximately, 5% of familial diffuse gastric cancer are due to large deletions in *CDH1* gene (15).In present study, several variants in *CDH1* gene reported among patients with hereditary and sporadic form of diffuse gastric cancer.

MATERIALS AND METHODS

Sampling

In this study 45 samples selected based on the histopathological criteria.17 cases are hereditary and 28 cases were sporadic form of diffuse gastric cancer. Patients included individual with DGC that referred to Al-Zahra hospital in Isfahan during the 2011 to 2015. DNA extraction was performed by *GeNet* Bio, Korea kit for blood samples and phenol chloroform method for formalin fixed paraffin-embedded samples. The exon/intron boundaries of *CDH1* gene were amplified by polymerase chain reaction (PCR).

DNA sequencing

All samples amplified by PCR and specific primers (available upon request). The PCR products were subject in sequencer instrument (Applied Biosystems/Life Technologies, Carlsbad, CA, USA).

RESULTS

Clinocopathologial characteristic of patients

The age average in hereditary and sporadic patients were 45.5 and 54.5 respectively.Hereditary patients include 7 male and 10 female and the sporadic patients include 20 male and 8 female. 17.6% cases of hereditary were stages I and II versus 82.4% stages III and IV. Sporadic cases were 21.4% in stages I and II against 67.9% stages III and IV and also 10.7% were unknown.

DNA sequencing

PCR products obtained from hereditary patients were displayed 7 variants and in sporadic patients were 3 variants. Variants in hereditary samples include c.348G>A, c.181A>G, c.2076T>C, c.2292C>T, c. 2331C>G, c.1177delA and c.889delA. In sporadic patients identified variants include c.348G>A, c.2076T>C and c.2253C>T. Comparative changes in hereditary and sporadic patients revealed that c.348G>A and c.2076T>C were same in both of them.

DISCUSSION

Most cases of gastric cancer are sporadic and familial aggregation can be seen only in 10% of cases. So, the hereditary diffuse gastric cancer make up a small number of cases(3).According to the diagnostic criteria by International Gastric Cancer Linkage Consortium (IGCLC), 15 to 50% patients with hereditary diffuse gastric cancer havegermline mutation in *CDH1* gene(16).Mutation rate of *CDH1* in DGC patients to 2010 were reported 50% (14), but using revised diagnostic criteria incidence rate reduce to 10-18% in low incidence country with gastric cancer (16). Some of the identified variants in present study were reported in previously studies that include c.2076T>C (17), n.2292C>T(18).Other identified variants in our study were new. Corso et al. were reported two mutations in Italian patients with DGC include p.Arg224Cys and C>A substitution in -63 position. Also, 5 of 21 patients were in stages I and II versus 15 in stages III and IV(19). Up to now only one study perform on an Iranian family with hereditary diffuse gastric cancer(20).In present study single amino acid substitution in hereditary patients were located on exons 1, 3, 7, 9, 13, 14 and 15 *CDH1*gene. Using bioinformatics software were displayed that p.777D>E amino acid substitution can negative effect on E-cadherin protein function. Exon 1 encoded for signal peptide domain and exon 3 encoding for propeptide part of E-cadherin protein. Exons 3, 9 and 13 were encoding extracellular domain of E-cadherin that necessary for cell adhesion and prevention of tumor invasion. Exons 14 and 15 were encoding for cytoplasmic domain of E-cadherin.Cytoplasmic domain binds to the β-catenin and suppressing this oncogene (20). Approximately 5% of *CDH1* are due to large deletions(15).Oliver et al. in 2009 were reported several deletions in exons 1, 2, 14, 15 and 16 *CDH1* gene(15).In other study large deletions in exons 7, 8 and 11 were reported (21). Asymptomatic carriers with *CDH1*germline mutation are at risk for diffuse gastric cancer. Also, *CDH1*germline mutation carriers are at risk for lobular breast cancer(8).On the other hand, no reliable screening tests to detect carriers. So, prophylactic total gastrectomy is recommended for *CDH1* gene mutation carriers.According to the results of this study, the age average of hereditary patients were 45.5 with 64.7% prevalence of mutations and the majority of patients were the advanced stages. The age average of the sporadic patients were 54.5 with 14.2% prevalence of mutation and the majority of patients were the advanced stages. Several different variants, including synonymous and non-synonymous substitution were reported in *CDH1* gene among hereditary and sporadic diffuse gastric cancer patients.Our results reveal that should be considered even one cases of diffuse gastric cancer with positive family history that prominence important regarding genetic counselling.

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