Pathology & Oncology Research (2020) 26:1057–1062
https://doi.org/10.1007/s12253-019-00658-5

ORIGINAL ARTICLE

Comparison of CDH1 Gene Hypermethylation Status in Blood and Serum among Gastric Cancer Patients

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Received: 28 November 2018 / Accepted: 1 April 2019 / Published online: 15 April 2019

Abstract

Hypermethylation is epigenetic alteration, well known for gene silencing. CHD1 gene is known as invasion and tumor suppressor gene, decreased expression due to hypermethylation could promote tumor cell invasion and metastasis. Present study designed to investigate the CDH1 gene promoter hypermethylation status by methylation specific polymerase chain reaction in 100 newly diagnosed gastric cancer patients. 53% of hypermethylation was observed in DNA extracted from blood in Gastric cancer patients while 66% was observed in serum based DNA. Significant differences in CDH1gene promoter hypermethylation was observed in serum based DNA extracted from Gastric cancer patients. Patients in early stage (I & II) vs advanced stage (III & IV), distant organ metastases vs no metastases had 60% vs 7% and 42% 24% of CDH1 promoter hypermethylation in serum DNA ($p = 0.006, 0.001$) respectively. Patients who were with lymph node invasion, loss of appetite, loss of weight had 55%, 47%, 61% CDH1 gene promoter hypermethylation compare to who were not with lymph node invasion, loss of appetite, loss of weight had 11%, 19%, 5% of hypermethylation and these differences was found to be significant. Strong association was observed with overall median survival of patients ($p < 0.0001$). Patients who had CDH1 gene promoter hypermethylation in serum based DNA showed poor overall median survival (14.3 months) and unmethylated patients had better overall median survival (33.2 months). CDH1 hypermethylation status was found to be associated with advancement of disease, distant organ metastases and lymph node invasion in Gastric cancer patients.

Keywords Gastric cancer · Hypermethylation · CDH1 gene · Prognosis

Introduction

Gastric cancer is one of the major widespread malignancy over the world. However, in the recent decades frequency in mortality rates has been reduced but it is still the 4th most common malignancy and the 2nd most important cause of cancer-related mortality globally [1]. Mostly gastric cancer patients diagnosed in advanced stage and individuals diagnosed with gastric cancer's 5 years of survival rate is only 20%–30%. Several environmental factors including Helicobacter *p*ylori infection is one of the major well-established reasons of gastric cancer. However, alterations at genetics level have been major cause for increased risk for gastric cancer [2]. Same as other cancer, gain-of-oncogenes function, loss of tumor suppressor genes function, accumulation of multiple epigenetic changes and genetic alteration involved in carcinogenesis. Tumor suppresser genes, promoter CpG hypermethylation causes gene silencing or down expression of gene participates in gastric carcinogenesis [3].

Methyltransferases are group of enzymes which add methyl group to 'CpG' islands of promoter region and it has been found that 'CpG' hypermethylation is restricted to cell to cell stages such as embryogenesis, adult cells development and differentiation [4]. However, increased methylatranferases activity was linked with CpG hypermethylation of tumor suppressor genes in human cancers [5]. E-dadherin or Cadherin-1 (*CDH1*) gene belongs to cell adhesion molecules family which located on q arm of chromosome number 16 (16q22.1) [6]. This protein has 3 domains, i.e. extracellular domain, transmembrane domain which linked to highly conserved cytoplasmic tail domain. It is essential for maintaining cellular differentiation, cell to cell linkage and which

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maintained normal characteristic of epithelial cells [7]. CDH1 gene expression was frequently lost or reduced in wide range of epithelial tumors that result in enhanced tumor invasion and distant organ metastases which has been found to be associated with several solid malignancies including Gastric cancer [8].

It has been reported that methylation profile of genes linked with different geographical location, different environmental exposure due to that reason different level of methylation pattern was observed with different ethnic groups [9]. Thus the present study aimed to investigate the hypermethylation status of CDH1 gene in Chinese Gastric cancer patients.

Materials and Methods

Patients Selection and Sample Collection Current study recruited histopathologicaly confirmed newly diagnosed 100 Gastric cancer patients as well as healthy controls. All the subjects recruited in current study were informed and written consent was obtained. The study was done at Medical section, Gansu Gem Flower Hospital and approved by the Institutional Ethics Committee. 4 ml of peripheral blood sample were drawn and 2 ml of blood were collected in EDTA and 2 ml of blood were collected in plain vials. Blood samples collected in EDTA vials were stored in -20 °C for DNA extraction and blood samples collected in plain vials were centrifuged at 1500 rpm for 10 min to collect the serum in tube. Further collected serum samples were stored at -20 °C for DNA extraction.

DNA Extraction and Bisulfite Modification Stored blood samples in EDTA were thawed and processed for whole blood DNA extraction using FlexiGene DNA extraction Kit (Cat No: 51206) by following manufacturer protocol. Serum stored in -20 °C were taken out at room temperature to thaw and then DNA was extracted using QIAamp Circulating Nucleic Acid kit (Cat No: 55114) following manufacturer protocol. Extracted DNA was subjected to bisulphite modification for hypermethylation study. Bisulfite modification of extracted DNA (1 μg) from whole blood and serum was carried out by using EpiTect Bisulfite Kits (Cat no: 59826) following manufacturer protocol and then immediately stored at -20 °C.

Methylation-Specific PCR After bisulfite conversion of DNA, Qualitative hypermethylation status *of CDH1* gene promoters CpG island was determined in all the subjects participated in study by methylation specific polymerase chain reaction (MS-PCR) using specific primers previously described by Kang et al. in 2003 is as (MF 5'- TTAGGTTAGAGGGTTATCGC GT-3'; MR 5'-TAACTAAAAATTCACCTACCGAC-3', UF 5'- TAATTTTAGGTTAGAGGGTTATTGT-3'; UR 5'-CACAACCAATCAACAACACA-3') [10]. PCRs were performed in 50 μl volume reaction, containing 4 μl bisulfite-modified DNA, 24 μl of 2x Hot Start PCR Mastermix (Fermentas), 0.50 μl forward primer (25 pm), 0.50 μl reverse primer (25 pm) and 21 μl nuclease free H_2O. The PCR programme was detailed as 94 °C for 10 min, 40 cycles at 95 °C for 40 s, primer annealing at 56 °C for 40 s, 72 °C for 40 s, final extension step at 72 °C 10 min and storage at 4 °C. The amplified PCR products were further electrophoresed on 2% agarose gels and evaluated under UV light to conferm amplification the hypermethylated (115 bp) and unmethylated (97 bp) amplification.

Statistical Analysis of Data

All the data were recorded in excel file and analysis was done by using SPSS 20.0 and Graph Pad Prism 5 version of software. Chi square and Fisher exact test were used to compare the different groups of variables. The Kaplan–Meier analysis was used to compute the overall survival of Gastric Cancer patients. p value <0.05 was considered to be statistically significant.

Results

Demographics All clinical and demographic characteristic of study subjects were depicted in Table 1. *In detail, t*otal 100 Gastric cancer patients were included in present study for CDH1 gene hypermethylation. Among Gastric cancer patients 65% patients were males and 35% patients were females. 31% patients were in age group of ≤50 years while 69% patients were in age group of >50 years. Patients who diagnosed in early stage of disease were 17% while 83% patients were in advanced stage of disease. It was observed that 52% patients had distant organ metastases while 48% patients were not showed distant organ metastases. However, more details were depicted in Table 1.

CDH1 Hypermethylation Status in Blood among Gastric Cancer Patients

It was observed that Gastric cancer patients showed overall 53% of CDH1 gene hypermethylation while 47% patients showed unmethylated CDH1gene in blood based DNA. It has been observed that male patients had 38% hypermethylation while females had 15% of CDH1 gene hypermethylation. Patients who were in age group of ≤50 years had 19% of hypermethylation while age group of >50 years had 34% of hypermethylation. Higher percentageof hypermethylation was observed in advanced stage of patients (45%) while in

Table 1 Clinical and demographic characteristic of gastric cancer patients

Variables	Gastric cancer patients No (%)
Total no. of cases	100 (100)
Gender	
Males	65 (65)
Females	35 (35)
Age (years)	
≤50	31 (31)
>50	69 (69)
TNM Stage	
Early Stage (I & II)	17 (17)
Stage (III & IV)	83 (83)
Distant Metastases	
Positive	52 (52)
Negative	48 (48)
Histopathology grade	
Well differentiated	20 (20)
Moderately differentiated	47 (47)
Poorly differentiated	33 (33)
Lymph node invasion	
Yes	71 (71)
No	29 (29)
Loss of appetite	
Yes	64 (64)
No	36 (36)
Loss of weight	
Yes	84 (84)
No	16 (16)

early stage it was only 6%. In detail, CDH1 gene promoter hypermethylation was mentioned in Table 2.

CDH1 Hypermethylation Status in Serum among Gastric Cancer Patients

CDH1 gene promoter hypermethylation status was observed in Gastric cancer patients and was found be associated with different variables. Overall 66% of Gastric cancer patients showed CDH1 promoter hypermethylation in DNA extracted from serum. It was observed that patients in advanced stage (III & IV) showed 60% CDH1 gene promoter hypermethylation compare to early stage (I & II) had 7% CDH1 gene promoter hypermethylation and differences was found to be significant ($p = 0.006$). Patients who had distant organ metastases had 42% of CDH1 gene promoter hypermethylation while patients without distant organ metastases showed 24% of CDH1 gene promoter hypermethylation and the differences was found to be significant ($p = 0.001$). Gastric cancer patients who has lymph node invasion, loss of appetite, Loss of weight had 55%, 47%, 61% CDH1 gene promoter hypermethylation while those who did not had lymph node invasion, loss of appetite, loss of weight showed 11%, 19%, 5% had CDH1 gene promoter hypermethylation and differences among them was found to be significant ($p = 0.0002$, $p = 0.03$, $p = 0.001$) respectively (Table 3).

Overall Survival of Gastric Cancer Patients with Respect to CDH1 Gene Hypermethylation Status in Blood

Overall survival of Gastric cancer patients were calculated on the basis of hypermethylation status evaluated in blood. Patients who showed CDH1 gene hypermethylated had 26 months of overall median survival while patients who did not had CDH1 gene hypermethylated showed better overall median survival (32.8 months) and differences was found to be border line significant ($p = 0.04$).

Overall Survival of Gastric Cancer Patients with Respect to CDH1 Gene Hypermethylation Status in Serum

Overall survival of Gastric cancer patients were calculated on the basis of hypermethylation status in serum (Figs. 1 and 2). Patients who showed CDH1 gene hypermethylated in serum had 14.3 months of overall median survival while patients who did not had CDH1 gene hypermethylated showed 33.2 months of overall median survival and differences among them was found to be significant ($p < 0.0001$).

Discussion

Promoter hypermethylation of tumor suppressor genes has emerged as a potential mechanism for the gene silencing and it is still under exploration in basic molecular biology, clinical oncology and in medical research [5]. In gastric cancer, aberrant hypermethylation was more frequently found to inactivate the genes than by genetic mutations [11].

Present research work evaluated the CDH1 gene promoter hypermethylation status in DNA extracted from whole blood and serum. We found significant association of serum based DNA with gastric cancer patients. However, no association was observed with variables among whole blood DNA extracted from Gastric cancer patients. Epigenetic alteration in Gastric cancer patients associated with disease advancement and spreads of disease. Patients who were in advanced stage of disease (60%) and had distant organ metastases (42%) showed higher level of CDH1 gene promoter hypermethylation were in serum based DNA was found to be significantly

Table 2 Association of CDH1 hypermethylation status in blood with different variables among Gastric cancer patients

Gastric cancer patients	CDH1 blood hypermethylation status		P value
	Yes, no (%)	No, no (%)	
Overall	53 (53)	47 (47)	–
Gender			
Male	38 (38)	27 (27)	0.13
Female	15 (15)	20 (20)	
Age (In year)			
≤ 50 years	19 (19)	12 (12)	0.26
> 50 years	34 (34)	35(35)	
Stage			
Early stage (I & II)	6 (6)	11 (11)	0.15
Advanced stage (III & IV)	45 (45)	38 (38)	
Metastases			
Yes	28 (28)	24 (24)	0.86
No	25 (25)	23 (23)	
Histopathological grade			
Well differentiated	11 (11)	9 (9)	0.81
Moderately differentiated	26 (26)	21(21)	
Poorly differentiated	16 (16)	17(17)	
Lymph node invasion			
Yes	39 (39)	32 (32)	0.54
No	14 (14)	15(15)	
Loss of appetite			
Yes	35 (35)	29 (29)	0.65
No	18 (18)	18(18)	
Loss of weight			
Yes	46 (46)	38 (38)	0.42
No	7 (7)	9 (9)	

associated ($p = 0.006$, $p = 0.001$). However, less percentage of CDH1 gene promoter hypermethylation were in advanced stage (45%) and distant organ metastases (28%) observed in blood based DNA. Positive association was observed with CDH1promoter hypermethylation with respect to lymph nodes invasion, loss of appetite and loss of weight. Gastric cancer patients who has lymph nodes invasion (55%), loss of appetite (47%), loss of weight (61%) showed higher percentage of CDH1 gene promoter hypermethylation in serum based DNA while less in blood based DNA (39%, 35%, 46%) respectively. Nawroz, H. et al. in 1996 and Hibi, K et al. in 1998 revealed that extracellular tumor derived DNA can be found in serum as well as in plasma [12, 13]. Kopreski M. S.et al. demonstrated that patients in early stage of cancer or disease showed detectable amount of extracellular circulating tumor DNA [14]. Patients with Sporadic Gastric cancer showed *CDH1* promoter hypermethylation ranges from 11% to 75% [15]. A study by Haroon R. et al. in 2016 found 65% of CDH1 promoter hypermethylation status in primary gastric cancer patients and positive correlation was also observed between CDH1 hypermethylation and metastasis in lymph nodes [16]. Study by Tamura et al. in 2000 suggested 51% of CDH1 gene hypermethylation as well as loss of expression by western blotting in primary gastric cancer patients [17]. Low CDH1 gene expression was found by promoter methylation in breast cancer patients and study was confirmed in cell lines as well. However, data is limited with tissues of primary breast carcinoma [18, 19]. 40.9% of CHD1 promoter methylation was reported in the primary breast cancer samples compared to matched normal tissues and decreased expression was also reported at mRNA level [20].

Strong and significant association of serum DNA based CDH1 gene promoter hypermethylation was observed with overall median survival of Gastric cancer patients. Patients with CDH1 promoter hypermethylation in serum showed very less overall median survival (14.3 months) while who did not have CDH1 promoter hypermethylation in serum showed 33.2 months of overall median survival. It suggested that serum based CDH1 gene promoter methylated patients showed around 19 months of higher overall survival than CDH1 gene promoter hypermethylated patients. However, blood DNA based CHD1 gene hypermethylation was not highly significant with overall median survival of Gastric cancer patients. It has been observed that CDH1 hypermethylation causes

Table 3 Association of CDH1 hypermethylation status in serum with different variables among Gastric cancer patients

Gastric cancer patients	CDH1 serum hypermethylation status		p value
	Yes No (%)	No No (%)	
Overall	66 (66)	34 (34)	–
Gender			
Male	45 (45)	20 (20)	0.35
Female	21 (21)	14 (14)	
Age (In year)			
≤ 50 years	21 (21)	10 (10)	0.80
> 50 years	45 (45)	24 (24)	
Stage			
Early stage (I & II)	7 (7)	11 (11)	0.006
Advanced stage (III & IV)	60 (60)	23 (23)	
Metastases			
Yes	42 (42)	10 (10)	0.001
No	24 (24)	24 (24)	
Histopathological grade			
Well differentiated	10 (10)	10 (10)	0.15
Moderately differentiated	31 (31)	16 (16)	
Poorly differentiated	25 (25)	8 (8)	
Lymph node invasion			
Yes	55 (55)	16 (16)	0.0002
No	11 (11)	18 (18)	
Loss of appetite			
Yes	47 (47)	17 (17)	0.03
No	19 (19)	17 (17)	
Loss of weight			
Yes	61 (61)	23 (23)	0.001
No	5 (5)	11 (11)	

reduced expression or loss of expression which has been found to be associated with decrease patient's survival [21].

Study from Korea by Lee et al. in 2003 reported that the CpG methylation status of CDH1 gene was linked with poor overall survival of hepato-cellular carcinoma [22].

Thus the current study reveals that CDH1 gene promoter hypermethylation status in serum based DNA was associated with advancement of disease, distant organ metastases and lymph node invasion in Gastric cancer patients. It was observed that CDH1 gene promoter hypermethylation was found

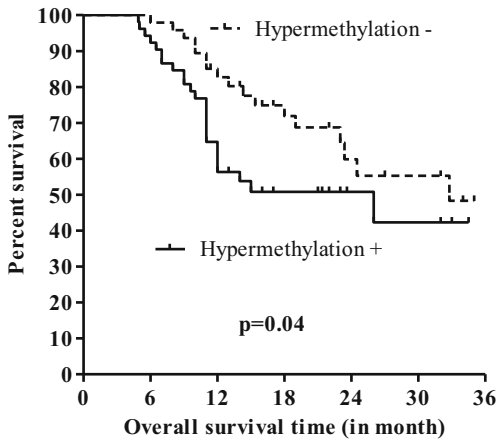

Fig. 1 Association of blood CDH1 gene promoter hypermethylation status with overall survival of Gastric cancer patients

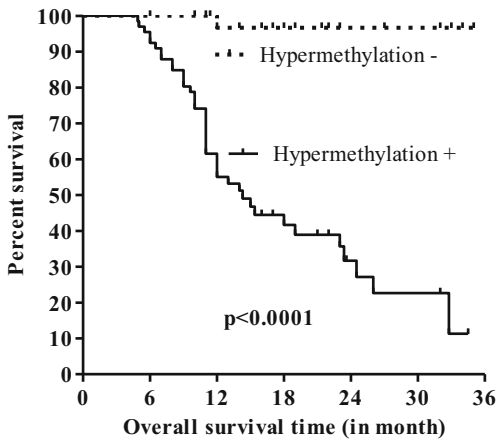

Fig. 2 Association of serum CDH1 gene promoter hypermethylation status with overall survival of Gastric cancer patients

to be is associated with worse overall survival of patients and this gene could be used as prognostic indicator for disease.

Conclusions Present study provides valuable data to predict the strong association of serum based DNA hypermethylation status in Gastric cancer patients. CDH1 gene promoter hypermethylation status in serum based DNA was associated with advancement of disease, distant organ metastases and lymph node invasion in Gastric cancer patients.

Acknowledgements Author thanks to all the study subjects that participated in this study.

Compliance with Ethical Standards

Conflict of Interest None.

ZL: Conducting experiment, Data collection and writing.

ZG: Concept, study design, analysis of data, drafting of manuscript and approval for final draft.

References

1. Siegel R, Ma J, Zou Z, Jemal A (2014) Cancer statistics, 2014. CA Cancer J Clin 64:9–29
2. Lee JH, Han SU, Cho H, Jennings B, Gerrard B, Dean M, Schmidt L, Zbar B, Vande Woude GF (2000) A novel germ line juxtamembrane Met mutation in human gastric cancer. Oncogene 19(43):4947–4953
3. Yousuf A, Bhat MY, Pandith AA, Afroze D, Khan NP, Alam K, Shah P, Shah MA, Mudassar S (2014) MGMT gene silencing by promoter hypermethylation in gastric cancer in a high incidence area. Cell Oncol 37:245–252
4. Goffin J, Eisenhauer E (2002) DNA methyltransferase inhibitors—state of the art. Ann Oncol 13:1699–1716
5. Baylin SB, Belinsky SA, Herman JG (2000) Aberrant methylation of gene promoters in cancer. Concepts, misconcepts and promise. J Natl Cancer Inst 92:1460–1461
6. Van RF (2014) Beyond E-cadherin: roles of other cadherin superfamily members in cancer. Nat Rev Cancer 14:121–134
7. Karayiannakis AJ, Syrigos KN, Chatzigianni E, Papanikolaou S, Alexiou D, Kalahanis N et al (1998) Aberrant E-cadherin expression associated with loss of differentiation and advanced stage in human pancreatic cancer. Anticancer Res 18:4177–4180
8. Humar B, Blair V, Charlton A, More H, Martin I, Guilford P (2009) E-cadherin deficiency initiates gastric signet-ring cell carcinoma in mice and man. Cancer Res 69:2050–2056
9. Galanter JM, Christopher RG, Sam SO, Torgerson D, Pino-Yanes M, Thakur N et al (2015) Methylation analysis reveals fundamental differences between ethnicity and genetic ancestry. Bio Rxiv. https://doi.org/10.1101/036822
10. Kang GH, Lee HJ, Hwang KS, Lee S, Kim JH, Kim JS (2003) Aberrant CpG Island hypermethylation of chronic gastritis, in relation to aging, gender, intestinal metaplasia, and chronic inflammation. Am J Pathol 163:1551–1556
11. Choi IS, Wu TT (2005) Epigenetic alterations in gastric Carcinogenesis. Cell Res 15:247–254
12. Nawroz H, Koch W, Anker P, Stroun M, Sidransky D (1996) Microsatellite alterations in serum DNA of head and neck cancer patients. Nat Med 2(9):1035–1037
13. Hibi K, Robinson CR, Booker S, Wu L, Hamilton SR, Sidransky D, Jen J (1998) Molecular detection of genetic alterations in the serum of colorectal cancer patients. Cancer Res 58:1405–1407
14. Kopreski MS, Benko FA, Borys DJ, Khan A, Kwee CM, McGarrity TJ et al (1998) Mutated KRAS DNA in plasma as a marker in early colorectal neoplasia. Proc Am Soc Clin Oncol 17:269
15. Lee TL, Leung WK, Chan MW, Ng EK, Tong JH, Lo KW, Chung SC, Sung JJ, To KF (2002) Detection of gene promoter hypermethylation in the tumor and serum of patients with gastric carcinoma. Clin Cancer Res 8:1761–1766
16. Rashid H, Alam K, Afroze D, Yousuf A, Banday M, Kawoosa F (2016) Hypermethylation status of E-Cadherin gene in gastric cancer patients in a high incidence area. APJCP 17(6):2757
17. Tamura G, Yin J, Wang S, Fleisher AS, Abraham JM, Kong D (2000) E-Cadherin gene promoter hypermethylation in primary human gastric carcinomas. J Natl Cancer Inst 92:569–573
18. Benton G, Crooke E, George J (2009) Laminin-1 induces E-cadherin expression in 3-dimensional cultured breast cancer cells by inhibiting DNA methyltransferase 1 and reversing promoter methylation status. FASEB J 23:3884–3895
19. Cheng CW, Wu PE, Yu JC, Huang CS, Yue CT, Wu CW, Shen CY (2001) Mechanisms of inactivation of E-cadherin in breast carcinoma: modification of the two-hit hypothesis of tumor suppressor gene. Oncogene 20:3814–3823
20. Liu J, Sun X, qin S, Wang H, Ning D, Li Y et al (2016) CDH1 promoter methylation correlates with decreased gene expression and poor prognosis in patients with breast cancer. Oncol Lett 11: 2635–2643
21. Caldeira JR, Prando EC, Quevedo FC, Neto FA, Rainho CA, Rogatto SR (2006) CDH1 promoter hypermethylation and E-cadherin protein expression in infiltrating breast cancer. BMC Cancer 6:48
22. Lee S, Lee HJ, Kim JH, Lee HS, Jang JJ, Kang GH (2003) Aberrant CpG island hypermethylation along multistep hepatocarcinogenesis. Am J Pathol 163(4):1371–1378