ejpmr, 2017,4(1), 05-08

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH
www.ejpmr.com

Research Article
ISSN 2394-3211
EJPMR

SEPT9; A PROMISING BLOOD-BASE BIOMARKER FOR EARLY DETECTION OF COLORECTAL CANCER.

Sussan Farmand-Rad[1], Ashraf Karbasi[1], Mahmood Tavallaei[2]*, Somayeh Chavoshei[2], Hasan Ashoori[2] Ghasem Azizi Tabesh[3]

[1]Baqiyatallah Research Center of Gastroentrology and Liver Disease. Baqiyatallah University of Medical Sciences, Tehran, Iran.
[2]Human Genetics Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
[3]Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Corresponding Author: Mahmood Tavallaei
Human Genetics Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Article Received on 24/10/2016 **Article Revised on 14/11/2016** **Article Accepted on 04/12/2016**

ABSTRACT
Background: Colorectal cancer (CRC) is the fourth most frequent cancer worldwide. CRC screening could reduce cancer-related mortality. The aim of this study was evaluation of septin9 methylated DNA for detection of CRC and precancerous colorectal lesions. **Methods:** Plasma samples were collected from 25 untreated CRC patients, 20 polyposis adenoma and 42 healthy control subjects before colonoscopy. We used methylation-specific PCR to assay the methylation status of septin9. **Results:** Septin9 was hypermethylated in 64% of CRC patients and 15% of polyposis adenoma. Sensitivity and specificity of septin9 methylated DNA test was 64% (95% CI: %43-%83) and 83% (95% CI: 0/68- 0/98) respectively. **Conclusion**: Results of this study suggest that septin9 is a valuable biomarker for CRC screening.

KEYWORD: septin9, colorectal cancer, methylation, screening test.

INTRODUCTION
Colorectal cancer (CRC) is the fourth common cancer worldwide with increasing rate of mortality and morbidity.[1] The estimated one million new CRC cases are diagnosed each year especially in developing countries.[2] A sufficient strategy for better management and increasing survival rate of patients is early diagnosis of CRC, such as other cancers.[3] Finding sensitive and specific biomarkers for early detection of CRC is required which in turn leads to decrease CRC-related mortality.[4] Different screening methods are used for detection of CRC in different populations.[5] These screening methods include fecal occult blood test, CT colonography, flexible sigmoidoscopy, colonoscopy and currently used methods such as molecular biomarkers.[6,7] Over the last decade, advances in genomics and molecular studies have demonstrated that different molecular pathways are involved in the development of CRC.[8] Epigenetic alternations in different oncogenes and tumor suppressor genes is an early changes in almost all types of cancer and is a promising field in screening and diagnosis of CRC.[9,10] One of these biomarkers, is plasma methylated Septin9 DNA (mSEPT9). SEPT9 is a member of septin gene family, and has been found to act as an oncogene and tumor suppressor gene in different types of cancer.[2,11] Several studies showed the role of SEPT9 in cancers, including leukemia, ovarian cancer, urologic cancer, brain tumors and CRC.[11,13] The molecular mechanism of SEPT9 in colon tumorigenesis is not well known yet.[14] Several studies have demonstrated that SEP9 is hypermethylated in CRC patients.[12,15,17] Based on these studies, the mSEPT9 looks a powerful marker for non-invasive detection of CRC. In this study SEPT9 methylation in plasma samples in healthy controls, CRC cases and patients with polyposis adenoma was assayed by Methylation Specific PCR (MSP) to evaluate the hypermethylation of this gene as a non-invasive biomarker for early detection of CRC.

MATERIAL AND METHODS
Study design and patients: A total of 42 healthy controls (no evidence of disease), 20 patients with polyposis adenoma (more than 1 cm diameter) and 25 patients with CRC (approved by histopathologic results) were enrolled in this study in Baqiyatallah hospital, Tehran, Iran. After obtaining written informed consent and questionnaire, 6 ml peripheral blood samples were collected from patients using K3EDTA vacutainer tubes (Gold vac™-China).

DNA extraction. Plasma samples were obtained from 6 ml freshly collected blood samples by centrifugation at 3000 rpm for 10 minutes. Plasma samples were then stored at -80°until further use. DNA isolation from 400 µL plasma was conducted using QIAamp DNA Blood

Mini kit (Qiagen-Germany), dissolved in 70 µL of sterile distilled water and stored at -20°C. Concentration of the isolated DNA was quantified using nanodrop spectrophotometer (Maestrogen-USA).

Bisulfite Treatment. 2 µg of extracted DNA was treated by sodium bisulphate to convert all unmethylated cytosins to uracils using the EZ DNA Methylation-Gold Kit (Zymo Research-USA). To confirm bisulfite DNA conversions, unmethylated and methylated human control DNA (Qiagen-Germany) was used as controls.

Amplication of mSEPT9. Methylation-Specific PCR (MSP) was performed to determine methylation status of SEPT9 gene. Primer sequences for the methylated and unmethylated templates are showed in Table 1. Each PCR reaction mix consisted of a total volume of 30 µl containing 15 µl hot start Taq Master Mix, 1 µM concentration of each primer, 1 µl DMSO and 3 µl bisulfite- modified DNA and 9 µl RNase free water.

Table 1. primers designed for methylated and unmethylated SEPT9 gene.

SEPT9 gene	sequence (5`>3`)
M_Forward primer	TAGTTATTGGCGTTAGCGCG
M-Reverse Primer	TACGCTACGCTACACCTAAC
U_Forward primer	TTGGGAAGTGTGGTGATTTTTG
U_Reverse primer	ACCCCTACACTACACTACAC

M: Methylated. Un: Unmethylated.

STATISTICAL ANALYSIS

Statistical analysis was conducted using the SPSS20 software (version 17.0, SPSS Inc, Chicago, USA). The Fisher's exact test and $\chi 2$ test were applied to study the statistical relationships between either MSP status and pathological or demographical results as well as evaluation of hypermethylation in patients. *P* value less than 0.05 was considered significant.

RESULTS

Patients. The study population consists of 87 individuals in three groups (CRC, polyposis adenoma and healthy controls). There was no significant difference in age, gender and weight between study groups (Table 2).

Table 2. Demographic characteristics of the study population.

Characteristics	Controls	polyposis	CRC	p value
Subject	42	20	25	-
Age (Mean)	55.6	63.5	64.5	0.5
BMI (Mean)	26.4	26.3	25.7	0.1
Smoking	No	35(83.3%)	14(70%)	19(76%)
Yes	7 (16.7%)	6 (30%)	6 (24%)	0.8

Methylation of SEPT9 in plasma samples

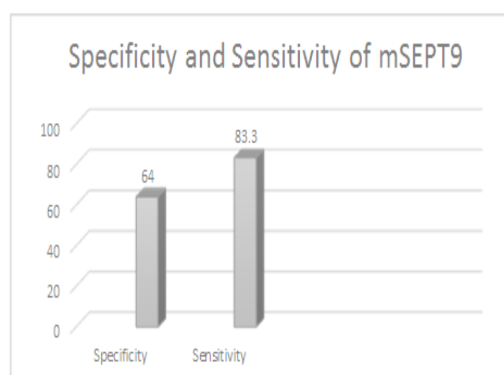
As shown in table 3, 7 of 42 (16%) healthy controls contained hypermethylated SEP9. In CRC group the rate of hypermethylation is 16 of 25 (64%) and in polyposis adenoma group, 3 of 20 (15%) showed hypermethylation in SEPT9.

Table 3. Methylation status of SEPT9 in study population

Group	Negative	Positive	p.value
Controls	35 (84%)	7 (16%)	0.002
Polyposis	17 (85%)	3 (15%)	0.007
CRC	9 (36%)	16 (64%)	0.001

Specificity and Sensitivity of mSEPT9

Evaluation of methylation status of SEPT9 in this study demonstrated that specificity and sensitivity of this gene is 64% and 83.3% respectively (figure 1).



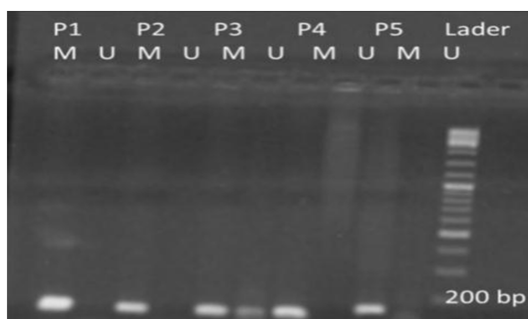


Figure 2. SEPT9 Methylation Status in affected CRC patients using methylated and unmethylated primers on 1.5% agarose gel. M: methylated. U: unmethylated. P1-5: patients.

DISCUSSION

Colorectal cancer is still one of the most commonly diagnosed cancers worldwide.[18] and although survival after diagnosis has improved over the last decades the overall 5-year survival remains less than 50%.[19] According to importance of early diagnosis, it seems that screening is therefore the only sufficient method of early detection.[20] Based on several studies, DNA markers in stool, blood and other body specimens have been investigated and different DNA panels have been developed as a powerful screening approach.[20,22] For example, In our pervious study, it has been concluded that detection of simultaneous hypermethylation of SPG20/ITGA4/ALX4 gene promoters is a specific and sensitive biomarker panel in the diagnosis of patients with cancer risk and can be helpful before colonoscopy.[23] The aim of our present study was evaluation of mSEPT9 in plasma samples of CRC patients in comparison with healthy controls. The rate of hypermethylation in CRC patients was 64% while in polyposis adenoma and healthy controls 15% and 16% showed hypermethylation in SEPT9 respectively. Numerous studies have been evaluated SEPT9 as a biomarker in diagnosis and prognosis of CRC. Grutzmann et al conducted a study on methylation of SEPT9 in 354 plasma samples and concluded that detection of mSEPT in plasma is a minimally invasive screening method.[16] In a study conducted by Tänzer et al, 73% CRC patients showed hypermethylation in SEPT9 while in healthy control group was only 9%.[15] In another study, Warren et al concluded that SEPT9 could be a specific marker for CRC screening instead of colonoscopy.[12] Toth el al showed SEPT9 methylation in 8.3% of controls, 30.8% of adenoma and 88.2% of CRC in a study using Real-time PCR in plasma samples.[13] Overall, these studies demonstrated that mSEPT9 is a valuable biomarker in detection of CRC as a molecular screening method.

In this study, specificity and sensitivity of SEPT9 test was 64% and 83.3% respectively. In other studies, the rate of specificity and sensitivity is approximately similar. Sensitivity of SEPT9 test in plasma samples of 50 CRC patients in Warren et al study was 88%.[12] 73.3% in David A et al study.[24] and 68% in Potter et al study.[14]

CONCLUSION

Collectively, results of this study in comparison with other aligned researches demonstrated that evaluation of mSEPT9 seems to be a reliable biomarker for early detection of CRC and we hope future researches make it an efficient and powerful method for CRC screening.

ACKNOWLEDGMENT

This study was approved and funded by the Vice Chancellor in charge of research, Baqiyatallah University of Medical Sciences and Baqiyatallah Human Genetic Research center. We would like to thank Colonoscopy center of Baqiyatallah hospital (Tehran, Iran).

REFERENCES

1. Naini MA, Mokarram P, Kavousipour S, Zare N, Atapour A, Zarin MH, et al. Sensitive and Noninvasive Detection of Aberrant SFRP2 and MGMT-B Methylation in Iranian Patients with Colon Polyps. Asian Pacific Journal of Cancer Prevention, 2016; 17(4): 2185-93.
2. Su XL, Wang YF, Li SJ, Zhang F, Cui HW. High methylation of the SEPT9 gene in Chinese colorectal cancer patients. Genetics and molecular research : GMR, 2014; 13(2): 2513-20.
3. Ladabaum U, Allen J, Wandell M, Ramsey S. Colorectal cancer screening with blood-based biomarkers: cost-effectiveness of methylated septin9 DNA versus current strategies. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2013; 22(9): 1567-76.
4. Ladabaum U, Alvarez-Osorio L, Rosch T, Brueggenjuergen B. Cost-effectiveness of colorectal cancer screening in Germany: current endoscopic and fecal testing strategies versus plasma methylated Septin9 DNA. Endoscopy international open. 2014; 2(2): E96-E104.
5. A stool DNA test (Cologuard) for colorectal cancer screening. Jama. 2014; 312(23): 2566.
6. Ausch C, Kim YH, Tsuchiya KD, Dzieciatkowski S, Washington MK, Paraskeva C, et al. Comparative analysis of PCR-based biomarker assay methods for colorectal polyp detection from fecal DNA. Clinical chemistry. 2009; 55(8): 1559-63.
7. Karley DG, D Tiwari, A. Biomarker for Cancer: A great Promise for Future. World J Oncol. 2011.
8. Koinuma K, Kaneda R, Toyota M, Yamashita Y, Takada S, Choi YL, et al. Screening for genomic fragments that are methylated specifically in colorectal carcinoma with a methylated MLH1 promoter. Carcinogenesis. 2005; 26(12): 2078-85.
9. Coppede F, Lopomo A, Spisni R, Migliore L. Genetic and epigenetic biomarkers for diagnosis, prognosis and treatment of colorectal cancer. World

journal of gastroenterology : WJG. 2014; 20(4): 943-56.
10. 10. Yang E, Kang HJ, Koh KH, Rhee H, Kim NK, Kim H. Frequent inactivation of SPARC by promoter hypermethylation in colon cancers. International journal of cancer Journal international du cancer. 2007; 121(3): 567-75.
11. Ravegnini G, Zolezzi Moraga JM, Maffei F, Musti M, Zenesini C, Simeon V, et al. Simultaneous Analysis of SEPT9 Promoter Methylation Status, Micronuclei Frequency, and Folate-Related Gene Polymorphisms: The Potential for a Novel Blood-Based Colorectal Cancer Biomarker. International journal of molecular sciences. 2015; 16(12): 28486-97.
12. Warren JD, Xiong W, Bunker AM, Vaughn CP, Furtado LV, Roberts WL, et al. Septin9 methylated DNA is a sensitive and specific blood test for colorectal cancer. BMC medicine. 2011; 9: 133.
13. Toth K, Wasserkort R, Sipos F, Kalmar A, Wichmann B, Leiszter K, et al. Detection of methylated septin9 in tissue and plasma of colorectal patients with neoplasia and the relationship to the amount of circulating cell-free DNA. PloS one. 2014; 9(12): 115415.
14. Potter NT, Hurban P, White MN, Whitlock KD, Lofton-Day CE, Tetzner R, et al. Validation of a real-time PCR-based qualitative assay for the detection of methylated SEPT9 DNA in human plasma. Clinical chemistry. 2014; 60(9): 1183-91.
15. Ng IOL, Tänzer M, Balluff B, Distler J, Hale K, Leodolter A, et al. Performance of Epigenetic Markers SEPT9 and ALX4 in Plasma for Detection of Colorectal Precancerous Lesions. PloS one. 2010; 5(2): 9061.
16. Grutzmann R, Molnar B, Pilarsky C, Habermann JK, Schlag PM, Saeger HD, et al. Sensitive detection of colorectal cancer in peripheral blood by septin9 DNA methylation assay. PloS one. 2008; 3(11): 3759.
17. Church TR, Wandell M, Lofton-Day C, Mongin SJ, Burger M, Payne SR, et al. Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. Gut. 2014; 63(2): 317-25.
18. Bogaert J, H p. Molecular genetics of colorectal cancer. Annals of Gastroenterology. 2014; 27.
19. Dolatkhah R, Somi MH, Bonyadi MJ, Asvadi Kermani I, Farassati F, Dastgiri S. Colorectal cancer in iran: molecular epidemiology and screening strategies. Journal of cancer epidemiology. 2015; 2015: 643020.
20. Kahi CJ, Anderson JC, Rex DK. Screening and surveillance for colorectal cancer: state of the art. Gastrointest Endosc. 2013; 77(3): 335-50.
21. Ashktorab H, Daremipouran M, Goel A, Varma S, Leavitt R, Sun X, et al. DNA methylome profiling identifies novel methylated genes in African American patients with colorectal neoplasia. Epigenetics: official journal of the DNA Methylation Society. 2014; 9(4): 503-12.
22. Imperiale TF, Ransohoff DF, Itzkowitz SH, Levin TR, Lavin P, Lidgard GP, et al. Multitarget stool DNA testing for colorectal-cancer screening. The New England journal of medicine. 2014; 370(14): 1287-97.
23. Rezvaneh Foroughi SC, Hasan Ashoori, Shahla Mohammad Ganji, Ghasem Azizi Tabesh, Tavallaei SF-RaM. EVALUATION OF NEW EPIGENETIC MARKERS SPG20, ITGA4 AND ALX4 IN PLASMA OF COLORECTAL CANCER PATIENTS. ejpmr. 3(11): 184-9.
24. David A, Johnson, Robert L, Barclay, Mergener K, G W, et al. Plasma Septin9 versus Fecal Immunochemical Testing for Colorectal Cancer Screening: A Prospective Multicenter Study. PloS one. 2014; 9(6): 1-8.