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THREE BIOMARKERS TO EARLY DETECT COLORECTAL CANCER

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ABSTRACT

Objective: Unusual methylation is one of the most frequent epigenetic modifications that can add to tumor development. Cell-free DNA can start from tumor tissue; in this manner, assessing methylation markers in cell-free DNA can be a promising strategy for malignancy screening. Our point was to find a strategy by comparing many studies to gather, to find promising biomarkers. Septin-9 gene (SEPT9), Syndecan-2 gene (SDC2) and Secreted frizzled-related protein 1 gene (SFRP1) are suitable biomarkers with different degrees of sensitivity and specificity for early detection of Colorectal cancer (CRC) by plasma sample. **Materials and Methods**: This literature review focuses on 2016 to 2020 for biomarkers and CRC early detection. Many websites were used, like PubMed, WHO, nature, and google scholar. The termers used in the research were marker or biomarker, SEPT9, Syndecan-2 gene (SDC2) and Secreted frizzled-related protein 1 gene (SFRP1) biomarkers, CRC diagnosis or screening, and colorectal or colon or rectal cancer. Many markers and methods have different sensitivity and specificity rates. **Results**: SEPT9, SDC2, and SFRP1 are promising biomarkers for the early detection of CRC. The combination of one or two biomarkers will increase the sensitivity and specificity detection rates. **Conclusion**: The outcomes from this review are that SEPT9, SDC2, and SFRP1 are promising biomarkers for detecting CRC. The combination between methylation biomarkers and traditional laboratory tests for CRC detection will increase the sensitivity and specificity. Our recommendation is to do these tests Epi pro Colon test, Colo Defense test, and Illumina human methylation test for CRC early detection in Saudi Arabia for patients from 30-40 years old and risk grope peoples. In this way, SEPT9, SDC2, and SEFR1 can turn into an incredible, for early CRC screening of high sensitivity and specificity.

KEYWORDS: Septin-9 gene (SEPT9), Syndecan-2 gene (SDC2), Secreted frizzled-related protein 1 gene (SFRP1), Colorectal cancer (CRC).

1. INTRODUCTION

The Colorectal cancer (CRC) is a leading source of death around the world.[1] Regular screening, early identification, and early treatment of CRC can accomplish effective avoidance and cure. Despite this 60% to 70% of CRC patients are not analyzed until late stages, and just 11.8% of the cases identified ahead of beginning stages.[2] No essential preventive measure has demonstrated adequacy in lessening occurrence; however, early discovery through screening has decreased mortality.[3] These days there is debate about which test ought to be utilized for CRC screening. Until the additional proof is gathered, current European guidelines accept the occult blood test followed by corroborative colonoscopy, which is helpful when resettable adenomas are recognized.[4] Most population-based screening programs utilize guaiac-based fecal mysterious blood test, which biochemically distinguishes little hints of blood from draining sores in dung, or fecal immunological test, which depends on immunodetection of human hemoglobin in feces. Most population-based screening programs utilize guaiac-based fecal mysterious blood test, which biochemically distinguishes little hints of blood from draining sores in dung, or fecal immunological test, which depends on immunodetection of human hemoglobin in feces. These tests have an affectability of 80% for CRC and 28% for adenoma.1 cm, and specificities in the scope 91 to 94%.[5] Besides, tolerant consistency with stool-based measures will in general be low.[6] Regula, J. et al. (2006) utilize colonoscopy as the best quality level for CRC screening is doubtful. It has detailed higher affectability (97%) and particularity (98%) for early recognition of CRC; however, the necessity of exceptionally prepared staff; awkward entrail planning; obtrusiveness; the danger of bleakness and mortality joined to the methodology.[7] Besides, in nations where public colonoscopy screening is accessible, consistency has regularly been low.[8] Serum-based markers would be profoundly appealing for CRC screening since they are negligibly obtrusive and could be incorporated in any standard wellbeing test without the need for extra stool inspecting, consequently expanding acceptance among patients. Current molecular biology test procedures permit a simpler age of

numerous theories biomarkers for analysis, visualization, or remedial reaction in CRC. Yet, the requirement for an appropriate approval has been frequently revealed.[9] The hidden idea is that tumor cells of CRC, even in its preinvasive stages, endure significant genetic alterations that release proteins or nucleic acids possibly discernible in organic liquids got by non-obtrusive techniques, for example, blood or feces.[10] Identification by molecular biology methods of these substances will fill in as a biomarker of illness to create indicative tests with the improved prescient force of current screening tests. In this manner, the point of the present investigation was to distinguish and approve new serum markers and exhibit their realizable value for early analysis of colon disease.[11]

Fecal occult blood tests (FOBT), colonoscopy, and carcinoembryonic antigens (CEA) are now accessible for CRC screening. FOBT is broadly utilized due to its low cost and non-invasiveness; however, the false positive rate is generally high. The fecal immunochemical test (FIT) has incredibly improved affectability for CRC contrasted and FOBT (fluctuated from 33% to 79%), prompting lower positive predictive values (PPVs). Interestingly, the consistency for colonoscopy is a low direct result of its obtrusiveness, cost, and danger of complexities. CEA is a serum biomarker broadly utilized for CRC discovery with a sensitivity of 40.9% to 51.8% and specificity of 85.2% to 95% for CRC detection.[12,13] However, it is more sensitive for late-stage CRC than the beginning stage. CRC thusly is utilized chiefly for assessment of the therapeutic effect of cancer recurrence, as opposed to as a marker for CRC early detection. Due to the high incidence of CRC, it is feasible to find markers to detect the early stage of carcinoma. Therefore plasma-based biomarkers like Septin-9 gene (SEPT9), SDC2, and SFRP1were chosen. By looking at the literature hoping for finding close to markers that help in CRC early detection.

2. METHODOLOGY

This literature review focus on the early detection of CRC by gathering the most cited articles researching biomarkers for CRC. Many articles have been published on an old date about CRC for a long time, and there are books, journals, and research groups working on CRC on different levels, genetics, and clinical levels. Our focus on studies has high sensitivity and specificity and valuable CRC early detection. This literature review focus on 2016 to 2020 for biomarkers and CRC early detection. Many websites were used, like PubMed, WHO, nature, and google scholar. Also, termers are used in the research marker or biomarker, screening biomarkers, and colorectal or colon or rectal cancer. Many markers and methods have different sensitivity and specificity rates, and we focus on the most sensitive and specific biomarkers for CRC early detection like SEPT9, SDC2, and SFRP1 biomarkers. We will choose three biomarkers to research it further and utilize them in this literature review hoping that we will find a suitable biomarker for CRC early detection.

3. RESULTS AND FINDINGS

3.1 CRC Screening

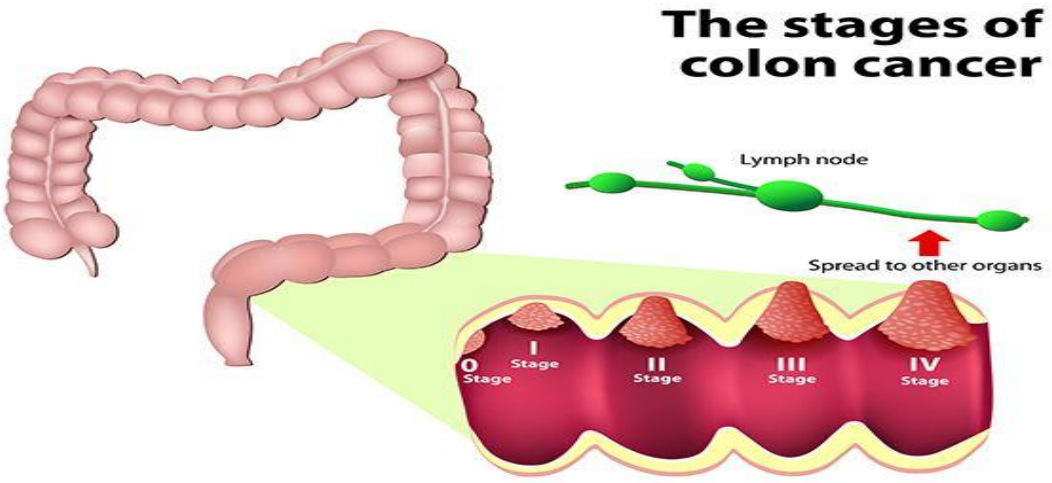

Figure 1 Stages of colorectal cancer.

Stages of colorectal cancer growth (CRC). The improvement of CRC usually starts with a non-malignant development shaping dysplastic tissue. Further cell development (hyperproliferation) results in the formation of a (benign) polyp or adenoma (Stage 0). A modest number of adenomatous polyps become dangerous, and an adenocarcinoma that attacks the muscular is Propecia develops (Stage I). Continuous development prompts increased tumor volume and further tissue invasion into the serosa (Stage II) and visceral peritoneum (Stage III). Finally, tumor cells can cause metastasis by blood and lymphatic vessels (Stage IV). Credit: Advances in colorectal cancer growth research, National Institutes of Health (NIH).[19]

3.2 the Epi pro Colon, Colo Defense test and Illumina human methylation test

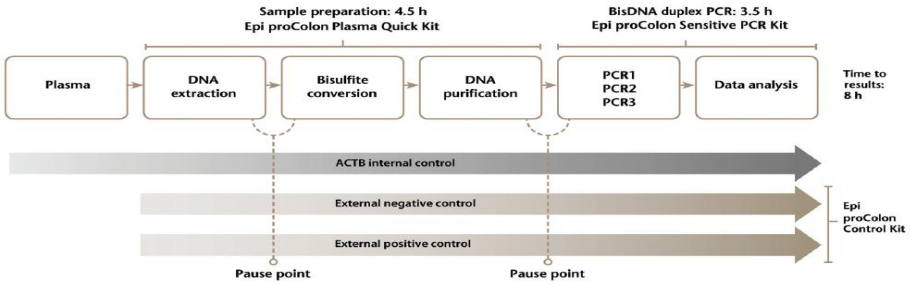

Figure 2 Epi pro Colon test workflow.

Outline of the Epi pro Colon workflow. Fully optimized, 30 patient samples plus a positive and negative control can be analyzed in a single run on the 7500 Fast Dx. Pause points indicate hard stops where samples can be stored to provide workflow flexibility.[39]

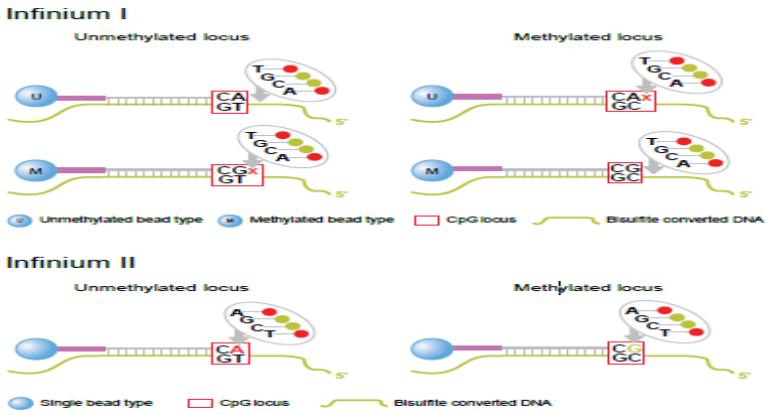

Figure 3: Illumina human methylation test.

The Human Methylation450 BeadChip utilizes both Infinium I and Infinium II tests, upgrading it is the expansiveness of coverage. Infinium I examine configuration utilizes two bead types for every Cytosines phosphate guanine (CpG) locus, each for the methylated and unmethylated states. The Infinium II plan utilizes one bead type, with the methylated form determined at the single base expansion extension after hybridization.[40]

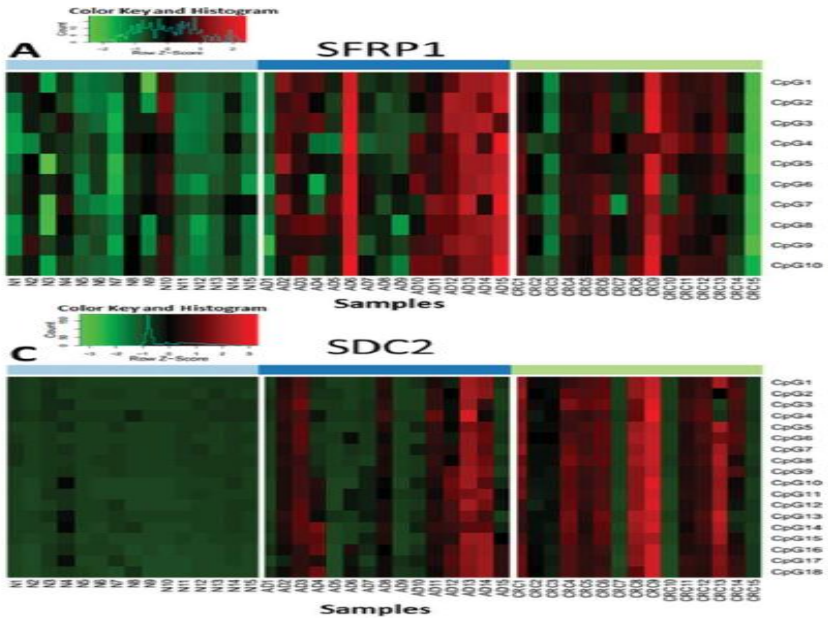

Figure 4: Illumina human methylation test result.

Methylation percentage data of selected CpG sites of the two markers were analyzed by pyrosequencing method. Heatmaps representing the methylation pattern of each CpG site of SFRP1 (A), SDC2 (C),) separately in CRC samples.[25]

3.3 Sensitivity and Specificity for SEPT9, SDS2, and SEFR1

Looking at the research papers, we found the sensitivity of SEPT9, As illustrated in the following table (table 1&2).

Table 1: Sensitivity and specificity for SEPT9.

Number of cases	Sensitivity	Specificity	Kit used
67 (33 CRC, 34 control)	82% (27/33)	88% (30/34)	Epi pro Colon 1.0
58 (34 CRC, 24 NED)	88.2% (30/34)	91.7% (22/24)	Epi pro Colon 2.0
Total 1544 (44 CRC, 1500 AA, small polyps, or NED	68.2% (30/44)	80.0% (adjusted by colonoscopy)	Epi pro Colon 2.0

AA=Advanced Adenoma; NAA=Non-Advanced Adenoma; NED=No Evidence of Diseases.[41]

Until now, many studies have demonstrated the viability of the test for CRC early detection and screening. Most of these studies were case-control examines, while one screening study was performed with a normal danger asymptomatic population. The sensitivity of the examiner went from 48.2% to 95.6% in the various path, with a high specificity somewhere in the range of 80.0% and 100.0%.[41]

Table 2: Sensitivity and specificity for SEPT9 and SDC2.

Stages	Serum volume (ml)	n	SDC2+ (%)	SEPT9+ (%)	SDC2 or SEPT9+ (%)
I	<1.5	3	66	33	100
	1.5-2.5	4	25	25	50
	>2.5	6	66	50	66.7
II	<1.5	16	26	75	81
	1.5-2.5	11	36	81.8	81
	>2.5	22	86	86.4	90
III	<1.5	10	90	70	100
	1.5-2.5	11	63	63	72.2
	>2.5	18	83.3	72	94.4
IV	<1.5	3	75	100	100
	1.5-2.5	4	100	100	100
	>2.5	NA	NA	NA	NA

Consequences of Colo Defense in recognizing CRC between various serum volumes. 225 serum tests after the blood biochemical test were assembled from the Associated Clinic of Xuzhou Clinical College, of which 111 were from CRC patients. The mean age of 61, and middle age of 62.[42]

The Colo Defense examination was utilized to measure methylation levels of SEPT9 and SDC2 qualities in 19 colorectal malignant growth tissues and matched neighboring para cancer tissues. The SEPT9 and SDC2 methylation levels were higher in 94.7% (18/19) and 100.0% (19/19) of threatening development tissues than in their combined precancer tissues, in this way making the Colo Defense test a technique for CRC screening. The chance of applying the Colo Defense test in CRC screening, 225 serum tests after the blood biochemical test was assembled, 111 were from CRC patients. The mean age of 61 and middle age of 62. The volumes of all serum tests went from 0.5 to 3.5 ml. The middle serum volumes for both CRC patients and NED people were 1.8 ml. When the serum tests were exposed to the Colo Defense test, the positive discovery rate for each phase of CRC appears in table 1. The sensitivity for recognizing CRC by methylated SEPT9 alone was 73.0%, with a specificity of 95.6%. The sensitivity by methylated SDC2 alone was 71.2%, with similar specificity of 95.6%. When methylated SEPT9 mixed with methylated SDC2 for secerning the CRC, the sensitivity was improved to 86.5% with a distinction of 92%. The methylated SEPT9 and methylated SDC2 board Colo Defense improved the high sensitivity and specificity of the test fair presentation in unmistakable CRC subjects from NED subjects. There was no expansion in the identification rate as the volume expanded for various stages when recognized by methylated SEPT9 alone, methylated SDC2 alone, or the methylated SEPT9 and methylated SDC2 board Colo Defense, and there appeared to be no massive distinction among the positive discovery paces of various volume groups.[42]

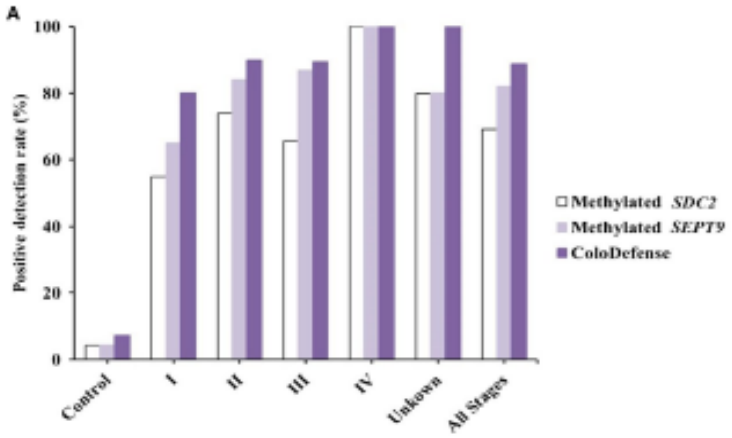

Figure 5 combination between SEPT9 and SDC2.

Affectability of Colo Defense test in identifying polyps and CRC across stages I-IV. A Positive recognition rate for control what not phases of CRC[43] (figure 5).

Colo Protection test for CRC screening, 384 plasma tests were assembled from patients of the Cooperated Center of Xuzhou Clinical College, of which 117 were from CRC patients, 23 from adenomas with high-grade dysplasia patients, 78 from patients with little polyps, and 166 from ordinary individuals. The times of all CRC patients went from 25 to 89, with a mean age of 61.8 and a middle-age of 63. The periods of typical people went from 21 to 69 with a mean age of 36.6 and a middle-age of 35 out of 117 CRC plasma tests whose stages were resolved dependent on the precisely resected examples, methylated SEPT9 was detected in 65.0% of stage I (13/20), 84.0% of stage II (42/50), 86.8% of stage III (33/38), 100% of stage IV (4/4), and 80.0% of obscure stage (4/5) examples. Methylated SDC2 was distinguished in 55.0% of stage I (11/20), 74.0% of stage II (37/50), 65.8% of stage III (25/38), 100.0% of stage IV.[43]

SFRP1 methylation has been analyzed broadly in CRC and is proposed as a non-intrusive biomarker for CRC screening. SFRP1 methylation in DNA from the serum of patients with CRC Sensitivity for methylated SFRP1 serum DNA (6% in patients with adenomas; and 67% in patients with CRC). However, serum SFRP1 methylation levels showed significantly higher particularity in CRCs (94%). SFRP1 gives a practical and great non-invasive screening tool for CRC and adenomas using blood tests. In a study that set out to determine SFRP1, Kumar, A. et al. (2019) found that SFRP1 is non-intrusive and more and simpler for specialists to perform than a colonoscopy. Studies have shown a repeat change from 52-95% in colorectal cancer growth and a higher rate of hypermethylation of SFRP1 in patients. The recurrence of methylation was not affected by the tumor histological subtype. In 84% of cases with lymph node metastasis, SFRP1 quality methylation was noted, which was important.[30]

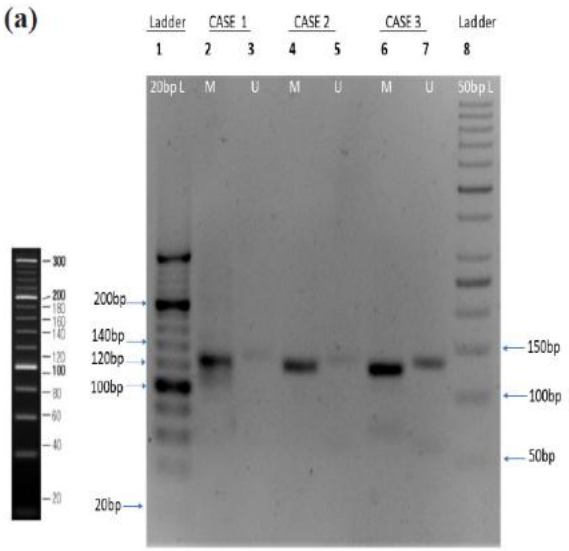

Figure 6: SFRP1.

Promoter Methylation Analysis of SFRP1 Gene in CRC by Methylation Specific Polymerase Chain Reaction (PCR) (figure 6). PCR amplified product run in 4.0% agarose gel. (a), Shows distinct band of a methylated fragment in lane 2,4,6 and their intensity are higher than respective unmethylated band lane 3,5,7 in 3 Colorectal carcinoma tumor tissue. 20bp ladder in lane 1 and 50bp ladder in lane 8.[30] Electrophoreses can be used as a confirmatory test for all these biomarkers.

4. DISCUSSION

4.1 CRC Screening

Numerous colorectal cancers can be prevented through standard screening. Screening can discover precancerous polyp's unusual developments in the colon or rectum with the goal that they can be eliminated before they transform into cancer. Screening is essential since, when discovered early, colorectal cancer is exceptionally treatable. Beginning phases of colorectal cancer usually present no indications, which will generally show up as the disease advances[14] see (figure 1). The American Cancer Society suggests that individuals at normal risk of colorectal malignant growth start standard screening at age 45. This should be possible either with a delicate test that searches for indications of malignant growth in an individual's stool (a stool-based test) or with a test that takes a gander at the colon and rectum (a visual examination). These choices are recorded individuals who are healthy and with a future of over ten years should proceed with regular colorectal malignancy screening through the age of 75. For individuals ages 76 through 85, the choice to be screened ought to be founded on an individual's feelings, overall health, and earlier screening history Individuals more than 85 should presently do colorectal malignancy screening.[15] Chinese Society of Gastroenterology (2014) Given its huge public populace and orderly assets usage issues, the Chinese Society of Gastroenterology agreement does not suggest colonoscopy as the first line evaluating test for risk people. The rules propose that people between ages 50 and 74 go through FOBT; furthermore, a survey is utilized to recognize high-risk factors.

The immunoassay FOBT ought to be liked over a compound FOBT, be that as it may, the rules moreover propose FOBT followed by FIT can be used.[16] Spanish Society of Clinical Oncology (SEOM) suggests evaluating normal-risk people between ages 50 and 74. Regular FOBT is suggested because of excellent proof (grade A), with FIT considered as the favored test. As an option to FIT, yearly or biennial high affectability FOBT rehashed at regular intervals or colonoscopy rehashed every ten years can be utilized (grade B nature of proof). National Guidelines for Colorectal Cancer Screening in Saudi Arabia (2015): The Saudi public standards disseminated by a working group of trained professionals suggest evaluating CRC in normal risk individuals starting at age 45, considering a center public time of CRC assurance of 55 for women and 60 for men. Screening people at 70 is not suggested (because of low-quality evidence) on account of the dangers of complications. However, it is referenced that specific people could benefit by screening after age 70,[16] WHO dispatched the "Guide to cancer early diagnosis," which means to help strategy creators and program supervisors encourage convenient determination and improve admittance to disease treatment for all. By creating viable techniques to identify cancer growth early, lives can be saved, and the individual, social and financial expenses of disease care are decreased.[17] The US Preventive Services task force discovered sufficient proof that was evaluating for colorectal disease with stool tests, colonoscopy, CT colonography, or adaptable sigmoidoscopy in ages 45 to 49 years gives a moderate advantage regarding decreasing colorectal malignant growth mortality and expanding life-years acquired. Although no investigations report on the benefits of screening clearly in those younger than 50 years, a few examinations detailing a relationship of less CRC passing with screening colonoscopy and decreased CRC mortality with screening FOBT included patients younger than 50 years displaying examinations propose more life-years are acquired. Less colorectal cancer deaths occur when screening starts at age 45 versus 50 years.[18]

4.2 SEPT9 methylation, SDC2, and SFRP1

Septin-9 is a protein encoded SEPT9 gene associated with a great range of organic pathways, for example, cytoplasm division, cell polarization, vesicle transport, and film recreation. This protein also acts as a tumor silencer since it effectively directs cell development and prevents uncontrolled cell division. In 2017, Issa, I. A. et al. published a paper abut methylation of SEPT9 at explicit Cytosines phosphate guanine (CpG) islands in the advertiser area is joined by quality quieting, with ensuing loss of malignant gene silencer. Because of the demonstrated job of SEPT9 downregulation in obsessive change from considerate to dangerous injuries in colorectal tissue. SEPT9 advertiser has been established to be methylated in colorectal disease tissue compared and by the normal colonic mucosa.[20,21] Syndecan-2 is a protein that in people is encoded by the SDC2 gene. syndecan-2 (SDC2) methylation was featured as an expected marker for early CRC detection. The syndecan-2 protein is an essential film protein associated with cell multiplication, cell transfer, and communication among cell migration. A DNA microarray investigation of neoplastic examples showed a high SDC2 methylation pace of around 95% regardless of the malignant growth stage.[22] Secreted frizzled-related protein 1 encoded by SFRP1 gene affect cell growth also, it is situated at chromosome 8p12-p11.1, inside a commonly deleted locale related with the improvement of numerous human tumors late examinations have exhibited down-regulated of SFRP1 in CRC utilizing semiquantitative analysis by constant polymerase chain response PCR, showed that SFRP1 mRNA articulation was down-regulated in CRC cases.[23,24] A sum of 44 CpGs situated in the chosen portions of the SFRP1 and SDC2 advertiser districts

were examined utilizing bisulfite pyrosequencing. The methylation example of each CpG site was resolved independently and looked at between the clinical groups. Addressing the methylation rates dependent on pyrosequencing CRC tests has appeared. Because SFRP1 broke down, the CpG site showed essentially expanded methylation in adenoma tissue, except for one CpG site that was discovered to be hypermethylated in adenoma CRC versus normal.

Further, 6 CpG destinations in the SDC2 advertiser showed essentially higher methylation rates in CRC tests contrasted and adenomas. The methylation difference of one CpG site CpG4 was significant just in CRC versus normal.[25] DNA methylation includes the addition of a methyl group to the carbon-5 position of the cytosine ring in the CpG dinucleotide and changing it over to methylcytosine. This interaction is catalyzed by DNA methyltransferase. In various tumors, the CpG islands of chose gene are defiantly methylated (hypermethylated), resulting in transcriptional suppression. This might be another component of gene inactivation.[26]

4.3 The application of SEPT9, SDC2, and SFRP1 assay in CRC early diagnosis

The application of a test in screening means that a test can be utilized freely as a technique for distinguishing high-risk people for certain diseases in population-based screening activities; confirming analysis is regularly required. At present, Many studies explore the performance of the SEPT9 test in different situations.[27] Dong W et al. 2016, says SEPT9 gene methylation was approved as a biomarker for (CRC) for more than ten years and accessible as the Epi pro Colon test as a guide in CRC detection for more than six years. It was demonstrated to be a precise, dependable, quick, and helpful molecular test. Screening study approved further worked on SEPT9 quality methylation examine in 1031 subjects in medical clinics. The sensitivity for CRC recognition was 76.6% at a specificity of 95.9%, and the outcomes showed a palatable discovery rate for each CRC stage, including beginning phases. The new SEPT9 examine, with upgraded simplicity, convenience, and lower cost, did not contrast in execution contrasted and Epi pro Colon 2.0, the popularized SEPT9 measure.[28] Kim et al. 2018, mention SDC2 methylation was featured as a possible marker for early CRC detection. They study SDC2 protein as an essential film protein associated with cell multiplication, cell migration, and interaction among cells and matrix.

A DNA microarray examination of neoplastic examples showed a high SDC2 methylation pace of roughly 95% regardless of the disease stage. SDC2 is frequently recognized in serum DNA obtained from CRC patients but infrequently in healthy subjects, showing potential as a biomarker for an early finding of CRC.[29] Further clinical approval utilizing blood from 131 CRC patients and 125 healthy people showed sensitivity and specificity of 87.0% and 95.2%, separately for distinguishing CRC. Critically, its high sensitivity of 92.3% for identifying stage I CRC recommends its likely efficiency for early CRC detection.[22] SFRP1 is fast becoming a key instrument in CRC early detection. SFRP1 is a tumor suppressor that assumes a significant part in regulating the multiplication and apoptosis of malignant growth cells. This gene is demonstrated to take an important role in colorectal disease improvement. Modified DNA methylation examples of this gene have effectively been accounted for in CRC tests SFRP1 gene in CRC patient.[1] Surveys such as that conducted by Kumar et al. 2019 have shown that frequently methylated in tumor tissue compared with non-tumor tissues. The recurrence of SFRP1 gene advertiser methylation in patients was 72.2%. Past investigations have shown a frequency going from 52-95% in colorectal disease. Few studies noticed a marginally higher frequency of hypermethylation of SFRP1.[30]

4.4 Advantages of SEPT9, SDC2, and SFRP1 assay in detecting CRC

SEPT9 examination is better than the fecal test inconsistency and comfort.[31] A particular component of the SEPT9 measure is that most investigations played out different PCRs to upgrade test sensitivity. Numerous studies have attempted to explain SEPT9 screening test gives an elective screening test to identify asymptomatic CRC in normal average-risk matured 50–75 years who have refused colonoscopy and fecal-based screening tests.[1] This test depends on detecting methylated SEPT9 as circulating tumor DNA in plasma using sensitive PCR. Song, L et al. (2017) investigated the differential impact of the SEPT9 test created after the revelation of variant hypermethylation at CpG island inside the SEPT9 gene in 97% of CRC.[32] However, much of the research has been that descriptive methylation was low or missing in normal colorectal mucosa.[33] The SEPT9 examination appeared to identify beginning phase CRC with high affectability, and it has unexpected favorable circumstances over serum protein markers in CRC early identification. Carcinoembryonic antigen CEA is the most usually utilized marker for CRC recognition. CEA has low affectability for beginning phase CRC, and it is even more generally used in post-medical procedure checking of CRC. SEPT9 methylation had the reserves to be the best blood-based single marker for CRC screening and early disclosure recently. Therefore, it seems that SEPT9 is a good option for CRC screening and early identification, as it has appeared to have higher consistency in CRC screening than fecal immunochemical FIT and colonoscopy scopy.[24]

In 2018, Ying Chen et al. published a paper describing methylated SDC2 new blood-based biomarker for CRC. SDC2 methylation frequently occurs on the all-CRC stages through complete methylation examination of CRC have recently revealed methylation DNA assay consisting of quantitative methylation-specific constant PCR for SDC2 methylation and showed that the SDC2 methylation test in DNA has high potential as an analytic

technique for early discovery of CRC.[22] A few examinations have announced that SDC2 methylation can be sensitively and specifically identified in blood tests from CRC patients.[34] Two further investigations detailed that the affectability of methylated SDC2 for CRC screening with serum or plasma tests was 87.0% with a specificity of 95.2%. Also, SDC2 alone showed higher positive detection rates for stages I and III CRC (53.9% and 79.5%).[35] A recent study by Li, H. et al. (2019) involved SFRP1 methylation in DNA from the serum of patients with CRC Affectability for methylated SFRP1 serum DNA (6% in patients with adenomas; and 67% in patients with CRC). However, serum SFRP1 methylation levels showed particularly higher CRC specificity (94%). SFRP1 provides a valuable and good non-invasive CRC screening tool using blood tests. The SFRP1 is more straightforward for the patients to follow and is simpler for doctors to perform than a colonocopy.[35,36]

4.5 How does the Epi pro Colon, Colo Defense test and Illumina human methylation test help in CRC detection

During cell turnover or apoptosis, genomic DNA is delivered into the circulatory system. The Epi pro Colon test targets circulating tumor DNA produced from tumor cells and depends on the subjective identification of methylated SEPT9 DNA in plasma. Abnormal methylation in the advertiser area of the SEPT9 gene is related to the event of CRC. In the Epi pro Colon test, without cell DNA cleaned from plasma is gotten through bisulfite change, a cycle for dissecting DNA methylation. In this way, using PCR with a fluorescent hydrolysis test empowers the subjective discovery of methylated SEPT9 DNA. Shirley, M et al. (2020) recognizes methylated cytosine-phosphate-guanine CpG destinations inside the v2 district of the SEPT9 gene. The continuous PCR test utilizes preliminaries focused on areas lacking CpG destinations. A blocker oligonucleotide that explicitly ties bisulfite changed over unmethylated arrangements inside the objective methylation-explicit fluorescent test, which only binds to and recognizes methylated sequences. Along these lines, in the plan, the measure explicitly emphasizes and identifies methylated groupings in the specific place.[37] Colo Defense test, work as Epi pro Colon a multiplex qPCR assay[38] (figure 2).

Methylation rate information of chosen CpG locales of the two markers dissected by pyrosequencing strategy. Heatmaps addressed the methylation example CpG site of SFRP1 (A), SDC2 (B), and independently in CRC tests utilizing a bisulfite pyrosequencing technique. Methylation levels on the shading scale are as the following: red: high methylation; dark: halfway methylation; green: low methylation level. Tests are introduced in sections. Clinical groping is shading coded on the top squares and addresses typical (light blue), adenoma (dull blue), and CRC (light green) tissue tests. CpG destinations have appeared in lines shown on each board's right side. Altogether higher methylation levels were noticed for 43 of the 44 contemplated CpG destinations situated in the advertiser locales of SFRP1 and SDC2, in CRC tests contrasted and sound controls, and 33 CpG destinations had raised methylation level in adenomas in contrast with typical examples: Promotion: adenoma; CRC: colorectal malignancy; CpG: cytosine phosphate guanine. These locales were examined utilizing Illumina Human Methylation 450K DNA methylation cluster information from The Disease Genome Chartbook (TCGA) data set. Methylation levels (b-estimations) of 99 CpG locales situated in the advertiser successions of SFRP1 and SDC2 qualities were resolved, Which showed a high methylation level on CRC patents[25] see (figure 2,3 & 4).

4.6 Sensitivity and Specificity for SEPT9, SDS2, and SEFR1

CRC disease is quite possibly the most predominant malignancies internationally, and there are different systems for CRC screening these days like colonoscopy, sigmoidoscopy, and FOBT for CRC screening. Colonoscopy has exhibited the most significant sensitivity and the most critical specificity for discovering colonic malignancies in the risk grope. However, it is a broad profession that has been restricted by certain disadvantages, such as post-polypectomy draining and holes, high cost, and a critical miss pace of colonic injuries, including enormous abnormalities. Circulating tumor DNA from malignancy is vital to those inspired by early malignancy detection. Unusual DNA methylation, viewed as a sign of malignancy, can be evaluated precisely in ctDNA. Therefore, DNA methylation testing in natural liquids addresses an excellent indicative instrument in dangerous diseases clinical administration. Epi pro-Colon2.0 measure, a blood ctDNA based SEPT9 methylation examine for CRC screening, has been endorsed by both the American FDA and Chinese FDA. It showed a sensitivity of 68.2% and specificity of 78.2%.[43] Zhao, G. et al. (2019) combine numerous biomarkers and strategies that have become popular in disease analysis and screening to improve sensitivity.[43] From our research, it is suggested that a combination of biomarkers will increase the detection rate. This strategy is a trend now, SEPT9 on an early stage for people with high risk. Our suggested policy makes screening test at 30 years old for the people with high-risk groups SEPT9+SDC2 as gulden test for detection will increase the sensitivity see (table 1,2 & figure 5). Also, the combination of methylated SEPT9+SFRP1+SDC2, additionally altogether, will increase the sensitivity higher than that of methylated SEPT9 alone. For the early stage of SEPT9 detection, different from the end stage, the degree of dictation increased gradually depending on the studies we reviewed see (figure 6). To improve the early-stage detection rate, we suggest following our suggested policy. There is some limitation like a small amount of cDNA in plasma at the beginning of the CRC and the amount of blood collected for the patent not less than 3

ml. Also, many advantages are fast, high sensitivity, and specificity. Disadvantages need more studies to show the accuracy and the need for highly trained people for this test. We recommend this biomarker to have more research and statistics by doing more studies and doing it on the clinical side to see how effective it is in real life. Biomarkers SEPT9, SDC2, and SFRP1, which are applied as a biomarker board, offer separate CRC patients from controls utilizing plasma tests giving a potential non-invasive analytic test. Utilizing numerous calculated relapse examinations, we recognized deeply and precise separation of CRC (91.5% sensitivity, 97.3% specificity) and adenoma (89.2% sensitivity and 86.5% specificity) plasma tests from sound controls in light of the methylation levels of the markers.[25]

5. CONCLUSIONS

In this literature looking for biomarkers to increase the sensitivity and specificity for screening CRC, we can apply in Saudi Ariba for CRC screening. Our literature review assessed new blood-based early CRC screening tests, Epi pro Colon, Colo Defense test, and Illumina human methylation test, distinguishing methylation biomarkers or genes SEPT9, SDC2, and SEFR1 for early detection of CRC. Our focus on studies had good results with high sensitivity and specificity. The articles we gathered have been published from 2016 to 2020 on many websites. The outcomes from this review SEPT9, SDC2, and SEFR1 are promising biomarkers for detecting CRC. It is a new area where we can do more studies to improve it. The combination between methylation biomarkers and traditional laboratory tests for CRC detection will increase the sensitivity and specificity. Our recommendation is to do these tests Epi pro Colon test, Colo Defense test, and Illumina human methylation test for CRC early detection in Saudi Arabia for patients from 30-40 years old and risk grope peoples. In this way, SEPT9, SDC2, and SEFR1 genes can be incredible for early CRC screening of high sensitivity and specificity.

Methylation biomarker non-invasive recognition of CRC from plasma tests samples. Plasma-based SEPT9 gene methylation was created as the Epi pro Colon test and was accounted for to be a promising strategy for the early recognition of CRC. This is supported in various clinical investigations, including case control. Furthermore, it shows that the methylated SEPT9 gene is a valuable biomarker for early CRC detection.[31] Also, Colo Defense for detecting methylated gens for SDC2 and SEPT9 may be a good tool for early detracting CRC. Finding a new strategy can also be applied to increase sensitivity and specificity by combining two or three biomarkers to gather. After combinations, we can assist the statistics and other relevant studies and these strategies for CRC early detection.

However, Maida, M. et al. (2017) say there is no universally agreed screening protocol for early disease stages, and critical variation remains. What is more, up to 70% of diseases introduced with symptoms are at an advanced stage. This emphasizes the value of screening programs with early detection of pre-malignant or early stage (I-II) CRC leading to improved CRC survival, quality of life, and disease-free outcomes. Moreover, screening for biomarkers at all stages, including diagnostic, prognostic, and predictive, may provide opportunities for targeted intervention to improve outcomes while reducing the risk of treatment toxicity.

6. REFERENCES

1. Ferlay, J., Parkin, D. M. & Steliarova-Foucher, E. Estimates of cancer incidence and mortality in Europe in 2008. *Eur. J. Cancer*, 2010; 46: 765–781.
2. Wu, D. *et al.* Detection of Colorectal Cancer Using a Simplified SEPT9 Gene Methylation Assay Is a Reliable Method for Opportunistic Screening. *J. Mol. Diagnostics*, 2016; 18: 535–545.
3. Burt, R. W. Colorectal cancer screening. *Current Opinion in Gastroenterology*, 2010; 26: 466–470.
4. Von Karsa, L. *et al.* European guidelines for quality assurance in colorectal cancer screening and diagnosis: Overview and introduction to the full Supplement publication. *Endoscopy*, 2013; 45: 51–59.
5. Parra-Blanco, A. *et al.* Diagnostic accuracy of immunochemical versus guaiac fecal occult blood tests for colorectal cancer screening. *J. Gastroenterol*, 2010; 45: 703–712.
6. Norton, J. D., Pawa, N., Arulampalam, T. & Norton, J. D. Screening for colorectal cancer: established and emerging modalities. *Nat. Rev. Gastroenterol. Hepatol*, 2011; 8: 711–722.
7. Regula, J. *et al.* Colonoscopy in Colorectal-Cancer Screening for Detection of Advanced Neoplasia. *N. Engl. J. Med.*, 2006; 355: 1863–1872.
8. Quintero, E. *et al.* Colonoscopy versus Fecal Immunochemical Testing in Colorectal-Cancer Screening. *N. Engl. J. Med.*, 2012; 366: 697–706.
9. Newton, K. & Hill, J. 5-FU and mismatch repair-deficient colorectal cancer: Is it time to consider a change in practice? *Color. Dis.*, 2010; 12: 706–707.
10. Ahlquist, D. A. Molecular Detection of Colorectal Neoplasia. *Gastroenterology*, 2010; 138: 2127–2139.
11. Miller, S. & Steele, S. Novel molecular screening approaches in colorectal cancer. *Journal of Surgical Oncology*, 2012; 105: 459–467.
12. Schreuders, E. H. *et al.* Colorectal cancer screening: A global overview of existing programmes. *Gut*, 2015; 64: 1637–1649.
13. Molnár, B., Tóth, K., Barták, B. K. & Tulassay, Z. Plasma methylated septin 9: A colorectal cancer screening marker. *Expert Review of Molecular Diagnostics*, 2015; 15: 171–184.
14. States, U. *et al.* Colorectal Cancer : The Importance of Screening and Early Detection.
15. Polyps, C. C. *et al. Colorectal Cancer Early Detection, Diagnosis, and Staging Can Colorectal Polyps and Cancer Be Found Early?* (2020).

16. Bnard, F., Brkun, A. N., Martel, M. & Von Renteln, D. Systematic review of colorectal cancer screening guidelines for average-risk adults: Summarizing the current global recommendations. *World J. Gastroenterol*, 2018; 24: 124–138.
17. World Health Organization. *Guide to Cancer - Guide to cancer early diagnosis*. *World Health Organization* (2017).
18. Cancer, C., Services, U. S. P. & Force, T. Mitigating Autogenous Shrinkage Ic Geiker and Bentz.Pdf., 2008; 149: 627–638.
19. Ii, S. Colorectal Cancer By Stage of Treatment Options Diagnosis, 501: 947831.
20. Grützmann, R. *et al.* Sensitive detection of colorectal cancer in peripheral blood by septin 9 DNA methylation assay. *PLoS One*. 2008; 3.
21. Issa, I. A. & NouredDine, M. Colorectal cancer screening: An updated review of the available options. *World Journal of Gastroenterology*, 2017; 23: 5086–5096.
22. Kim, J. H. & Park, S. C. Syndecan-2 Methylation as a New Biomarker for Early Detection of Colorectal Neoplasm, 2018; 12: 479–480.
23. Wang, Z., Li, R., He, Y. & Huang, S. Effects of secreted frizzled-related protein 1 on proliferation, migration, invasion, and apoptosis of colorectal cancer cells. *Cancer Cell Int.*, 2018; 18: 1–10.
24. Song, L., Jia, J., Peng, X., Xiao, W. & Li, Y. The performance of the SEPT9 gene methylation assay and a comparison with other CRC screening tests: A meta-analysis. *Sci. Rep.*, 2017; 7.
25. Barták, B. K. *et al.* Colorectal adenoma and cancer detection based on altered methylation pattern of SFRP1, SFRP2, SDC2, and PRIMA1 in plasma samples. *Epigenetics*, 2017; 12: 751–763.
26. Chim, C. S., Pang, R., Fung, T. K., Choi, C. L. & Liang, R. Epigenetic dysregulation of Wnt signaling pathway in multiple myeloma, 2007; 57: 2527–2536.
27. Church, T. R. *et al.* Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. *Gut*, 2014; 63: 317–325.
28. Wu, D. *et al.* Detection of Colorectal Cancer Using a Simpli fi ed SEPT9 Gene Methylation Assay Is a Reliable Method for Opportunistic Screening, 2016; 18.
29. Oh, T. J. *et al.* Feasibility of quantifying SDC2 methylation in stool DNA for early detection of colorectal cancer. *Clin. Epigenetics*, 2017; 9: 1–11.
30. Kumar, A., Gosipatala, S. B., Pandey, A. & Singh, P. Prognostic Relevance of SFRP1 Gene Promoter Methylation in Colorectal Carcinoma, 2019; 20: 1571–1577.
31. Wu, D. *et al.* Detection of Colorectal Cancer Using a Simplified SEPT9 Gene Methylation Assay Is a Reliable Method for Opportunistic Screening. *J. Mol. Diagnostics*, 2016; 18: 535–545.
32. Puccini, A. *et al.* Colorectal cancer: epigenetic alterations and their clinical implications HHS Public Access Author manuscript. *Biochim Biophys Acta*, 2017; 1868: 439–448.
33. Hitchins, M. P. *et al.* Methylated SEPTIN9 plasma test for colorectal cancer detection may be applicable to Lynch syndrome. *BMJ Open Gastroenterol*, 2019; 6: 1–11.
34. Han, Y. D. *et al.* Early detection of colorectal cancer based on presence of methylated syndecan-2 (SDC2) in stool DNA. *Clin. Epigenetics*, 2019; 11: 1–11.
35. Chen, Y. *et al.* Performance of a novel blood-based early colorectal cancer screening assay in remaining serum after the blood biochemical test. *Dis. Markers*, 2019; 2019.
36. Li, H. *et al.* Performance of a methylight assay for methylated sfrp2 dna detection in colorectal cancer tissue and serum. *Int. J. Biol. Markers*, 2019; 34: 54–59.
37. Shirley, M. Epi proColon® for Colorectal Cancer Screening: A Profile of Its Use in the USA. *Mol. Diagnosis Ther.*, 2020; 24: 497–503.
38. Zhao, G. *et al.* Aberrant DNA Methylation of SEPT9 and SDC2 in Stool Specimens as an Integrated Biomarker for Colorectal Cancer Early Detection. *Front. Genet*, 2020; 11: 1–8.
39. Potter, N. T. *et al.* Validation of a Real-Time PCR – Based Qualitative Assay for the Detection of Methylated SEPT9 DNA in Human Plasma RESULTS: 2014; 1191: 1183–1191.
40. Illumina. Infinium Human Methylation 450 data sheet. *Illumina* 3 (2012).
41. Song, L. & Li, Y. Methylated Sept9 Gene is a Sensitive Biomarker for all Stages of Colorectal Cancer. *Color. Cancer*, 2015; 1: 1–7.
42. Chen, Y. *et al.* Performance of a novel blood-based early colorectal cancer screening assay in remaining serum after the blood biochemical test. *Dis. Markers*, 2019; 2019.
43. Zhao, G. *et al.* Multiplex methylated DNA testing in plasma with high sensitivity and specificity for colorectal cancer screening. *Cancer Med.*, 2019; 8: 5619–5628.
44. Zhao, G. *et al.* A novel plasma based early colorectal cancer screening assay base on methylated SDC2 and SFRP2. *Clin. Chim. Acta*, 2020; 503: 84–89.