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Assessment of The Expression of Both knj12 and znf132 Genes as Potential Non-Invasive Plasma Markers for Early Detection of Different Colonic Neoplasia

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Abstract:

Background: Colorectal cancer (CRC) is one of the most prevalent cancers globally and a main cause of cancer-related death. Current diagnostic methods like colonoscopy, while effective, suffer from low patient compliance due to invasiveness. Hence, there is a critical need for sensitive and non-invasive biomarkers for early detection. Aim: This research aimed to assess the plasma expression levels of the knj12 and znf132 genes as potential non-invasive markers for early detection of different forms of colonic neoplasia. **Methods:** This prospective case-control research has been carried out on 80 participants. Group I included 55 patients with colonic polypoidal lesions (CRC, advanced adenoma, and serrated sessile adenoma), while Group II included 25 healthy controls. Gene expression was analyzed and correlated with lesion characteristics and histopathology. **Results:** There was a statistically significant positive association between knj12 and znf132 expression across all neoplastic subgroups. At cutoff values of 1.13 (knj12) and 1.05 (znf132), both genes demonstrated moderate sensitivity and specificity for CRC detection. ZNF132 showed higher diagnostic performance in serrated sessile adenomas (sensitivity 87%, specificity 60%). Combined testing improved early neoplasia detection and correlated with lesion histopathology and classification. **Conclusion:** KNJ12 and ZNF132 show promising potential as non-invasive plasma biomarkers for early detection of colonic neoplasia. Their combined evaluation could serve as a valuable adjunct to current screening strategies, especially for patients with low compliance to invasive procedures like colonoscopy.

Keywords: Colonic Neoplasia; knj12; znf132; Gene expression; Non-invasive biomarkers.

Introduction

Colorectal cancer (CRC) ranks as one of the three most prevalent cancer types and is the 2nd main cause of cancer-related mortality globally [1].

The disease burden can be mitigated by effective population-based screening techniques that identify precancerous lesions and early-stage cancer [2].

Screening tests enhance case prognosis and expect long-term survival by identifying cancers in their early stages, resulting in reduced CRC-related mortality rates [3].

Presently, colorectal cancer screening recommendations advocate for colonoscopy and fecal immunochemical test (FIT) as the 1st-tier options [4]. Nonetheless, the invasiveness, dietary restrictions, and troublesome bowel preparation associated with colonoscopy result in a reduced compliance rate [5].

The gold standard for colorectal cancer detection is sigmoidoscopy or colonoscopy, accompanied by biopsy for pathological confirmation of lesions [4].

The fecal occult blood test (FOBT)/FIT is a non-invasive, economical procedure that is easy to administer; nevertheless, it lacks adequate sensitivity for the identification of advanced adenomas (AAs) or stage I colorectal cancer [5].

The Food and Drug Administration (FDA) authorized a stool DNA-based colorectal screening test, Cologuard, for colorectal cancer screening in average-risk people. It exhibits overall CRC sensitivities ranging from 73.8% to 92.3% in comparison with colonoscopy as non-invasive options for CRC screening [6].

Aberrant regulation of gene expression by DNA methylation is a well-documented phenomenon in carcinogenesis and is frequently observed in tumor suppressor genes across several cancer types, such as colorectal cancer [7].

Elevated concentrations of circulating free methylation DNA in the bloodstream of cancer cases have been documented [7].

Numerous potential blood-based epigenetic indicators exhibiting elevated sensitivity and specificity for the early detection of colorectal cancer have been found in prior investigations; however, few have undergone comprehensive validation and are accessible commercially [8].

Epi proColon, a blood-based assay that assesses the methylation state of the Septin9 gene, is the sole blood-based test sanctioned by the FDA for colorectal cancer screening and has been utilized clinically for almost ten years [9].

The efficacy of Septin9 promoter methylation as a biomarker for colorectal cancer screening has been questioned because of its restricted sensitivity, particularly in early-stage tumors [9].

A model comprising previously reported markers, such as thbd, vav3-as1, itga4, alongside many additional markers as znf132, knj12, and sfmbt2, has found a unique colorectal cancer-specific methylation model at the tissue level, demonstrating significant sensitivity to early-stage colorectal cancer and stage dependency [8].

The knj12 gene is part of the inward-rectifier potassium channel family, which is crucial for the regulation of K+ channels. One investigation indicates that it may promote cancer cell proliferation via modulating RelA-activated NF-κB signaling [10].

Ion channel overexpression and NF-κB signaling overexpression have been documented in several tumors, such as colorectal carcinoma [10].

Methylation-mediated silencing events for znf132 have been confirmed in breast cancer and esophageal squamous cell carcinoma, but not in colon cancer [10].

The goal of this research was assessment of the expression of both knj12 and znf132 genes as potential non-invasive plasma markers for early detection of different colonic neoplasia.

Patients and Methods

This prospective cross-sectional study was conducted on 80 persons from Hepatology and Gastroenterology department inpatient and outpatient clinic at Benha university hospitals between June 2023 to June 2024.
The protocol of the research has been approved by the ethical committee of Benha University Hospitals, Benha University {M.D.17.2.2023}
An informed written consent was obtained from all patients participating in this study after explaining the study measures in details.

Patients:

The study cases were divided into 2 groups:

Group (I) included 55 patients who were had polypoidal colonic lesions including: Colorectal cancer: 25 patients, Advanced adenoma: 15 patients and Serrated sessile adenoma: 15 patients

Group (II) included 25 normal subjects as controls

Inclusion criteria: Aged above 18 years, Patients with colonic polypoidal lesions (Colorectal cancer – Advanced adenoma – Serrated sessile adenoma) and Normal subjects with no personal history of symptoms suggesting colorectal cancer, chronic illness or malignancy and has no family history of malignancies.

Exclusion criteria: Age below 18 years, Patients with other system malignancy and patients refused to be entitled in this study

Ethical Consideration

The data that were obtained from participants are confidential. The study participants were not identified by name in any report or publication concerning this study. Before the participants were admitted in this study, the purpose and nature of the study, as well as the risk–benefit assessment was explained to them. An informed consent was obtained**.**

Methods:

All patients were subjected to: Complete history taking including (personal history, chief complain, analysis of symptoms e.g. bleeding per rectum, altered bowel habit, weight loss, dysphagia, persistent vomiting, hematuria or chronic loin pain and unexplained microcytic anemia, past medical and surgical history and family history of CRC and neoplasia) Physical examinations including (general examination and vital signs to exclude systemic diseases: e.g. acromegaly, uncontrolled DM, chronic obstructive pulmonary diseases (COPD) related to smoking, inflammatory bowel diseases also signs of pallor, cyanosis, jaundice or lymph node enlargement). Abdominal examination with stress on alarm signs for CRC e.g. cachexia, pallor and exclusion of symptoms suggestive of malignancy of other systems e.g. breast lamp, breast skin changes were done.

Investigational Studies done to all patients were: Complete blood count, erythrocyte sedimentation rate, serum creatinine, alanine aminotransferase, aspartate aminotransferase, international normalized ratio, prothrombin time, serum albumin, serum bilirubin (total and direct), CA19-9, CEA and gene expression analysis using Real-Time PCR.

Colonoscopy Procedure

The colonoscopy was performed using Olympus CV 180 device. All patients underwent full colonoscopy. Bowel preparation was done, using oral magnesium citrate for 2 days prior to the procedure, or polyethylene glycol for 1 day prior to the procedure (depending on patient and physician preference). Patients fasted for 16-24 hours (water and fiber free juices were allowed), patients underwent 3 enemas using hypertonic solution of sodium phosphates the day before the procedure.

Polyps class was determined as regard Paris classification:

Ip: pedunculated polyp
Is: sessile polyp
IIa: slightly elevated polyp
IIb: flat polyp
IIc: slightly depressed polyp
III: ulcerated polyp

Gene Expression Analysis Using Real-Time PCR

To evaluate the expression levels of knj12 and znf132 genes, plasma samples were collected and processed using real-time PCR (RT-PCR) techniques.

RNA Extraction and Preparation

RNA was extracted from plasma using the GF-1 Blood Total RNA Extraction Kit (Vivantis, Malaysia). The quantification of RNA was performed using the HERA SYBR® Green qPCR Kit (Willowfort, UK). Prior to RNA purification, several reagents were prepared, including 80% ethanol, absolute ethanol (>95%), 2-mercaptoethanol, and enzymes such as lysozyme, lyticase, and zymolase, which are essential for bacterial or yeast RNA purification. Stability of the kit components was ensured up to 18 months from the manufacturing date.

RNA Purification Procedures

The RNA purification process was carried out entirely at room temperature, taking precautions to avoid RNase contamination.

Quantification Using HERA SYBR® Green qPCR Kit

The HERA SYBR® Green qPCR Kit is a highly sensitive method for DNA quantification and gene expression analysis. It includes HERA Hot Start Taq DNA Polymerase and SYBR Green qPCR buffer.

Statistical Analysis

The gathered data was processed, coded & analyzed utilizing the Statistical Package for Social Sciences (SPSS) version 26 for Windows® (IBM SPSS Inc, Chicago, IL, United States of America).

The sample size has been calculated utilizing STATA software (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC). By conducting sample size analysis for matched case control study, the expected AUC is 0.799 & 0.764 for kcnj12 & znf132 respectively, case to control ratio of 2:1, the sample size was 42 subjects, (28 diseased & 14 healthy subjects) at a power of 80% & alpha error of 5%.

Results

There was a statistically insignificant variance among the examined groups regarding age, sex, residence and comorbidity **(Table 1)**

There was a statistically insignificant variance among examined groups regarding CA 19-9, ALT, AST, Creatinine, WBC, PLT and INR while there was statistically significant variance among examined groups with regard o CEA, Bilirubin, Hemoglobin, ESR and Albumin **(Table 2)**

A statistically significant variance has been observed between studied groups with regard to KNJ 12 expression and ZNF132 expression **(Table 3 and figure 1)**

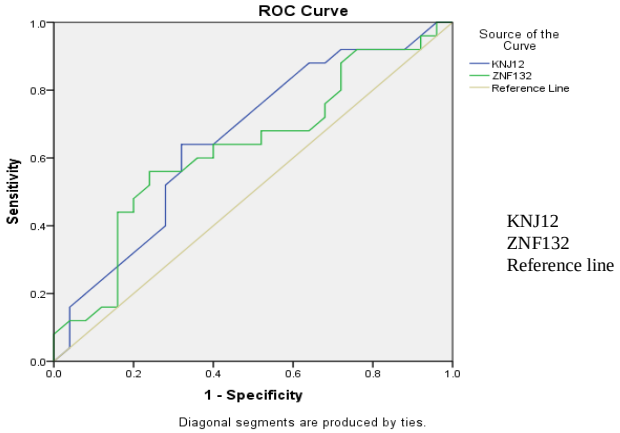

Figure (1): Rock analysis for prediction of incidence of colorectal cancer.

Table (1): Sociodemographic data characteristics among the studied groups:

	Colorectal cancer group N=25	Advanced adenoma group N=15	Serrated sessile adenoma group N=15	Control group N=25	P-value
Age					
Mean± SD	50.72±10.72	48.66±9.49	54.66±12.06	47.72±13.25	0.30
	N (%)	**N (%)**	**N (%)**	**N (%)**	
Sex					
Male	12 (48%)	10 (66.7%)	10 (66.7%)	19 (76%)	0.2
Female	13 (52%)	5 (33.3%)	5 (33.3%)	6 (24%)	
Residence					
Qalyobia (urban)	6 (24%)	4 (26.7%)	4 (26.7%)	6 (24%)	0.97
Qalyobia (Rural)	8 (32%)	4 (26.7%)	5 (33.3%)	9 (36%)	
Menofya (Urban)	2 (8%)	2 (13.3%)	0 (0%)	1 (4%)	
Menofya (Rural)	9 (36%)	5 (33.3%)	6 (40%)	9 (36%)	
Comorbidity					
DM	6 (24%)	5 (33.3%)	6 (40%)	5 (20%)	0.44
HTN	3 (12%)	4 (26.7%)	2 (13.3%)	4 (16%)	
DM + HTN	5 (20%)	0 (0%)	2 (13.3%)	2 (8%)	
CKD	0 (0%)	1 (6.7%)	0 (0%)	1 (4%)	
Bronchial asthma	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)	
No Comorbidities	11 (44%)	5 (33.3%)	4 (26.7%)	13 (52%)	

Table (2): Distribution of laboratory data among the studied groups:

	Colorectal cancer group N=25	Advanced adenoma group N=15	Serrated sessile adenoma group N=15	Control group N=25	P-value
CA 19-9 Mean± SD	57.97±141.16	10.86±6.20	6.88±5.04	3.33±1.20	0.07
CEA Mean± SD	34.52±33.64	4.89±2.83	4.20±2.08	4.27±4.12	**<0.001**
ALT Mean± SD	28.61±27.52	19.32±9.02	22.8٠±7.97	27.28±9.65	0.34
AST Mean± SD	29.56±18.17	23.60±9.18	26.26±7.91	31.2±8.91	0.25
Bilirubin Mean± SD	0.68±0.25	0.92±0.19	0.83±0.22	0.85±0.15	**<0.001**
Creatinine Mean± SD	0.94±0.20	0.95±0.16	0.93±0.16	0.92±0.18	0.95
WBC Mean± SD	7.80±2.12	7.26±1.61	7.74±1.48	7.61±2.00	0.84
Hemoglobin Mean± SD	9.86±1.66	10.97±1.93	11.70±1.92	11.41±1.98	**<0.001**
PLT Mean± SD	272.04±100.41	254.6±99.59	244±76.17	240.52±68.88	0.60
ESR Mean± SD	45.76±30.77	21.53±10.32	19.26±8.03	20.00±9.94	**<0.001**
INR Mean± SD	1.09±0.13	1.08±0.09	1.07±0.12	1.04±0.78	0.46
Albumin Mean± SD	3.38±0.60	4.15±0.64	4.23±0.62	4.17±0.57	**<0.001**

Table (3): Distribution of expression of both kcj12 and znf132 genes among the studied groups:

	Colorectal cancer group N=25	Advanced adenoma group N=15	Serrated sessile adenoma group N=15	Control group N=25	P-value
KNJ 12 expression (fold increase) Mean± SD	2.86±0.17	1.67±0.85	1.35±0.55	1.10±0.10	**<0.001**
ZNF 132 expression (fold increase) Mean± SD	1.39±0.06	1.18±0.21	1.13±0.13	1.05±0.12	**<0.001**

There was a positive statistically significant correlation in Colorectal cancer group between KNJ 12 expression and ZNF 132 expression, a positive statistically significant association has been observed between KNJ 12, ZNF 132 expression and histopathology and There was a positive statistically significant correlation between ZNF 132 expression and Length of lesion **(Table 4 and figure 2).**

There was a positive statistically significant correlation in Advanced adenoma group between KNJ 12 and ZNF 132 and There was a positive statistically significant correlation between KNJ 12 and ZNF 132 and Paris classification while A negative statistically significant correlation has been observed between KNJ 12 and ZNF 132 and size **(Table 4)**

There was a positive statistically significant correlation in serrated sessile adenoma group between KNJ 12 and ZNF 132 expression. While There was a negative statistically significant correlation between KNJ 12 and ZNF 132 expression and Paris classification, KNJ 12 and ZNF 132 expression and histopathology **(Table 4)**

At cutoff value 1.13 KNJ 12 had sensitivity of 70% and specificity of 50% with significance for prediction of incidence of colorectal cancer, and at cutoff value 1.05 ZNF 132 had sensitivity of 66% and specificity of 52% with significance for prediction of incidence of colorectal cancer **(Table 5 and figure 3).**

At cutoff value 1.126 KNJ 12 had sensitivity of 66% and specificity of 48% with significance for prediction of incidence of advanced adenoma, and at cutoff value 1.043 ZNF 132 had sensitivity of 73% and specificity of 44% with significance for prediction of incidence of advanced adenoma **(Table 5)**

At cutoff value 1.071 KNJ 12 had sensitivity of 53% and specificity of 40% with significance for prediction of incidence of serrated sessile adenoma, and at cutoff value 1.064 ZNF 132 had sensitivity of 87% and specificity of 60% with significance for prediction of incidence of serrated sessile adenoma **(Table 5)**

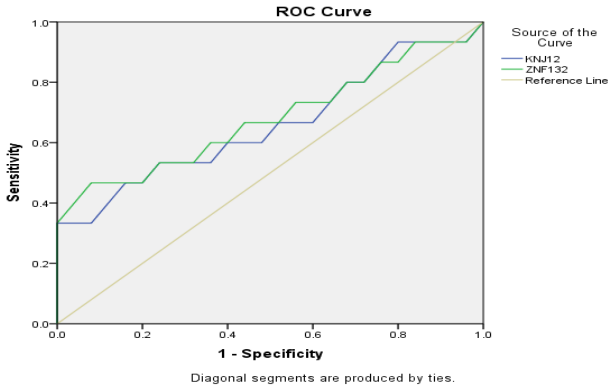

Figure (2): Rock analysis for prediction of incidence of advanced adenoma

Table (4): Correlation of KNJ 12 and ZNF 132 and other parameters in Colorectal cancer group, advanced adenoma group and sessile serrated adenoma group:

Colorectal cancer Group	ZNF 132 expression **R**	**P**	KNJ 12 expression **R**	**P**
KNJ 12 expression	0.022	0.918	1	-
ZNF 132 expression	1	-	0.022	0.918
Description of lesion	-0.260	0.209	-0.082	0.697
Histopathology	0.283	0.170	0.005	0.983
Length of lesion	**0.089**	**0.672**	**-0.292**	**0.157**
Advanced adenoma group	ZNF 132		KNJ 12	
	R	P	R	P
ZNF 132	1	-	.883**	**0.001**
KNJ 12	0.883**	**0.001**	1	-
Paris classification	0.210	0.453	0.149	0.596
Size	-0.166	0.553	-0.080	0.778
Sessile serrated adenoma group	ZNF 132 expression **R**	**P**	KNJ 12 expression **R**	**P**
ZNF 132 expression	1	-	0.761**	**0.001**
KNJ 12 expression	0.761**	**0.001**	1	-
Paris Classification	-0.148	0.598	-0.209	0.456
Histopathology	-0.296	0.284	-0.230	0.409

r: person correlation p: p value, p value ≤ 0.05 statically significant

Table (5): Diagnostic performance of KNJ 12 and ZNF 132 for prediction of incidence of colorectal cancer, advanced adenoma and sessile serrated adenoma.

Test Result Variable(s)	*Area under curve (AUC)*	*Cut off point*	*Sensitivity*	*Specificity*	*PPV*	*NPV*	*Std. Error*	*Asymptotic Sig*	*Asymptotic 95% Confidence Interval*	
									Lower Bound	***Upper Bound***
KNJ 12	***0.658***	***1.13***	***70%***	***50%***	***60%***	***65%***	***0.073***	***0.045***	***0.515***	***0.801***
ZNF 132	***0.665***	***1.05***	***66%***	***52%***	***58.6%***	***61.9%***	***0.072***	***0.037***	***0.523***	***0.807***
Test Result Variable(s)	*Area under curve (AUC)*	*Cut off point*	*Sensitivity*	*Specificity*	*PPV*	*NPV*	*Std. Error*	*Asymptotic Sig*	*Asymptotic 95% Confidence Interval*	
									Lower Bound	***Upper Bound***
KNJ 12	***.659***	***1.126***	***66%***	***48%***	***43.5%***	***70.6%***	***.096***	***.091***	***.471***	***.846***
ZNF 132	***.680***	***1.043***	***73%***	***44%***	***35.5%***	***55.5%***	***.095***	***.059***	***.493***	***.867***
Test Result Variable(s)	*Area under curve (AUC)*	*Cut off point*	*Sensitivity*	*Specificity*	*PPV*	*NPV*	*Std. Error*	*Asymptotic Sig*	*Asymptotic 95% Confidence Interval*	
									Lower Bound	***Upper Bound***
KNJ 12	***.528***	***1.071***	***53%***	***40%***	***34.8%***	***58.8%***	***.100***	***.769***	***.331***	***.725***
ZNF 132	***.773***	***1.064***	***87%***	***60%***	***56.5%***	***88.2%***	***.074***	***.004***	***628***	***.918***

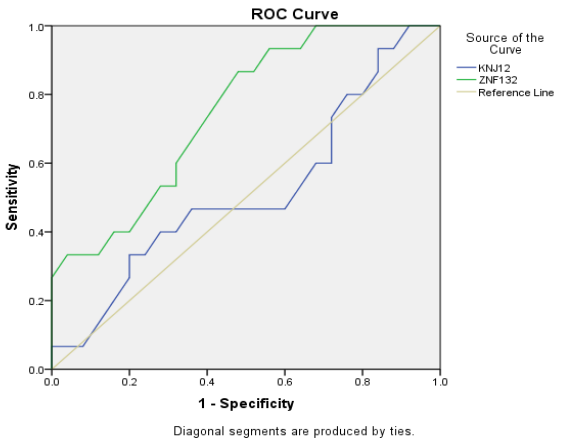

Figure (3): Rock analysis for prediction of incidence of Serrated sessile adenoma.

Discussion

CRC is among the three most common cancer types and the second leading cause of cancer related deaths worldwide [(1)]. Patient non-compliance and the compromised performance of FIT-based screening strategies limit their utility in current CRC screening market [(8)].

This study was designed to assess the expression of both kcnj12 and znf132 genes in colorectal cancer, precancerous lesions and healthy subjects.

There was a positive statistically significant correlation in CRC group between knj12 expression and ZNF 132 expression, there was a positive statistically significant correlation between knj12, ZNF 132 expression and histopathology and there was a positive statistically significant correlation between ZNF 132 expression and Length of lesion. This was not included in the studies done in our knowledge.

There was a positive statistically significant association in advanced adenoma group between knj12 and ZNF 132. This was in line with findings of Wan et al., [(11)].

There was a positive statistically significant association between knj12 and ZNF 132 and Paris classification while there was a negative statistically significant association between knj12 and ZNF 132 and size of the lesion. This is not included in the previous studies.

There was a positive statistically significant association in serrated sessile adenoma group between knj12 and ZNF 132 expression.

At cutoff value 1.13 knj12 had sensitivity of 70% and specificity of 50% with significance for prediction of incidence of colorectal cancer, and at cutoff value 1.05 ZNF 132 had sensitivity of 66% and specificity of 52% with significance for prediction of incidence of colorectal cancer.

Zhang et al., [(12)] observed that knj12 and znf132 exhibited superior efficacy in detecting colorectal cancer in an independent validation cohort (N = 69), achieving an overall AUC of 0.911 [ninety-five percent CI 0.834–0.988], a sensitivity of 0.800 [ninety-five percent CI 0.667–0.933], and a specificity of 0.971 [ninety-five percent CI 0.914–1.000].

At cutoff value 1.126 knj12 had sensitivity of 66% and specificity of 48% with significance for prediction of incidence of advanced adenoma, this was in line with findings of Wan et al., ([(11)] who noted that with sensitivity of 52.6%, specificity of 78.4% and AUC of 0.73 in the test set knj12 was a good predictor of incidence of advanced adenoma.

At cutoff value 1.043 ZNF 132 had sensitivity of 73% and specificity of 44% with significance for prediction of incidence of advanced adenoma.

Also, Zhang et al., [(12)]; Maqbool et al., [(13)] observed that, knj12 and ZNF132 were significant predictor of incidence of advanced adenoma.

At cutoff value 1.071 knj12 had sensitivity of 53% and specificity of 40% with significance for prediction of incidence of Serrated sessile adenoma, and at cutoff value 1.064 ZNF 132 had sensitivity of 87% and specificity of 60% with significance for prediction of incidence of Serrated sessile adenoma this coincides with a new study by Pu et al., [(14)] who recognized the methylation profiles of candidate zinc finger genes (ZFGs) as possible biomarkers for early CRC identification, especially for KRAS-mutated cases .

One of these ZFGs is ZNF132 (sensitivity eighty-three percent specificity ninety-seven percent, AUC = 0.93), demonstrated significantly greater diagnostic capabilities compared to SEPT9 (sensitivity eighty-three percent specificity eighty-seven percent, AUC = 0.91).

Our research corroborates a preceding study by Zhang et al. [(12)], in which both knj12 and znf132 exhibited superior efficacy in identifying colorectal cancer within an independent validation cohort (N

= 69), achieving an overall AUC of 0.911 [ninety-five percent CI 0.834–0.988], a sensitivity of 0.800 [ninety-five percent CI 0.667–0.933], and a specificity of 0.971 [ninety-five percent CI 0.914–1.000].

The stage-stratified sensitivity of the model was 0.455 [ninety-five percent CI 0.227–0.682], 0.667 [ninety-five percent CI 0.289–1.000], 0.800 [ninety-five percent CI 0.449–1.000], 0.800 [ninety-five percent CI 0.449–1.000] and 0.842 [ninety-five percent CI 0.678–1.000] for advanced adenoma and CRC stage I-IV, correspondingly.

However, Liu et al., [15] mentioned that there is limited reported demonstration of knj12 mutation or aberrant expression in colon tumor, whereas, as for znf132, methylation mediated silencing events have been confirmed in breast cancer and esophageal squamous cell carcinoma, nevertheless, not in colon malignance.

In a current investigation, Hamada et al. [16] focused on the znf132 gene, for which DNA hypermethylation throughout N to T transition (ΔMT–N) was significantly related to vascular invasion.

Conclusion

KNJ12 and ZNF132 appear to be promising non-invasive plasma biomarkers for the early detection of colonic neoplasia. Assessing them in combination may provide a useful complement to current screening approaches, particularly for individuals who are less likely to undergo invasive procedures such as colonoscopy.

Conflict of interest

The authors have no conflict of interest to declare.

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