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## **Welcome letter from Editor-in-Chief**



Welcome to the Int J Cancer and Biomedical Research (IJCBR)!

It is with great pleasure that I write this editorial to welcome you to the IJCBR. This journal provides a platform for publication of original and reviews research articles, short communications, letter to editor, thesis abstract, conference report, and case studies. These types of publication are directed at the interface of the fields of cancer and biomedical research.

The IJCBR relies on a distinguished expert of the Advisory and Editorial Board Members from the top international league covering in depth the related topics. They timely review all manuscripts and maintain highest standards of quality and scientific methodology and ethical concepts. Meanwhile, we take all possible means to keep the time of the publication process as short as possible.

I take this chance to welcome your contributions to the IJCBR and have every expectation that it will soon become one of the most respected journals in both the fields of cancer and biomedical research.

A handwritten signature in blue ink that reads "Mohamed L. Salem". The signature is fluid and cursive.

**Mohamed L. Salem,**

Editor in Chief

## Role of serum miR-21 and miR-92a in colorectal cancer diagnosis as novel molecular biomarkers

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### ABSTRACT

**Background:** Our study aimed to evaluate the clinical utility of detecting plasma microRNAs (miR-21 & miR-92a) for diagnosis of colorectal cancer patients and its relation to tumor staging. **Patients and Methods:** Quantitative real-time RT-PCR was applied to determine the relative expression level of miR-21 and miR-92a in serum. The sensitivity and specificity of these markers were evaluated by receiver operating characteristic (ROC) curve analysis. Final staging of colorectal cancer cases was assigned according to results of histopathologic examination of surgically resected specimens. **Results:** This study included 52 cases of colorectal cancer (CRC), 20 cases of precancerous colorectal lesions, and 20 healthy controls. Both Plasma miR-21 and Plasma miR-92a were significantly higher in CRC group compared to both the control group and precancerous group. Also, they were significantly higher in advanced CRC stages than early CRC stages. The sensitivity and specificity of miR-21 for discriminating CRC from controls were found to be 90.38% and 100.0%, respectively. However, for miR-92a, sensitivity and specificity were found to be 94.23% and 100.0%, respectively. For discriminating CRC cases from precancerous lesions, the sensitivity and specificity of miR-21 were found to be 75.08% and 95.0%, respectively. However, for miR-92a, sensitivity and specificity were found to be 80.77% and 100.0%, respectively. **Conclusions:** both plasma miR-21 and miR-92a have significant value for early detection of CRC as non-invasive screening molecular biomarkers with high sensitivity and specificity. They also help for differentiation between patients with benign and malignant colorectal lesions and those with early and advanced CRC.

**Keywords:** Biomarkers; Colorectal Cancer; Micrnas; miR-21; miR-92a

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### INTRODUCTION

The incidence of colorectal cancer (CRC) is globally increasing especially in the western world and now ranks at 3<sup>rd</sup> commonest cancer (Rawla et al., 2019). Late presentation and delayed diagnosis in a more advanced stage are associated with a very poor prognosis. Early diagnosis and initiation of therapy are associated with the best chances of cure. This can be reflected in a reduction of mortality from CRC (Keane and Johnson, 2012).

The main screening tools advocated by many surgeons for early detection of colorectal lesions are colonoscopy, CT colonography, and

barium enema. However, their widespread application is limited. This could be attributed to their high-cost and invasive nature (Brenner et al., 2014). Therefore, there is a great demand for a less invasive test for the early diagnosis of CRC (Zhang et al., 2018). Currently, there are few serum biomarkers used for screening or early detection of CRC with variable sensitivity and specificity levels (Herrerros-Villanueva et al., 2019).

MicroRNAs are small non-coding RNAs that have an important role in the regulation of cell differentiation, mitosis, apoptosis, and carcinogenesis (Croce and Calin, 2005; Aslam et al., 2009). They act at the posttranscriptional

level by binding to target mRNA, preventing its translation, or by transcriptional gene silencing (He and Hannon 2004; Hede 2005). Regarding carcinogenesis, microRNAs act as oncogenes or tumor-suppressor genes. They have a key role in cancer progression, including CRC (Calin et al., 2002; He et al., 2005; Schetter et al., 2008; Ng et al., 2009).

Certain microRNAs have been evaluated as biomarkers for CRC diagnosis. A few of them were selectively expressed in CRC, such as up-regulation of miR-21 and miR-92a and down-regulation of miR-422a (Clancy et al., 2015; Zekri et al., 2016). Only a few studies have evaluated the role of serum microRNAs as biomarkers for CRC diagnosis at an early stage with contradictory results (Schetter et al., 2008; Chen et al., 2013; Liu et al., 2013; Toiyama et al., 2013).

This study aimed to evaluate the clinical utility of detecting plasma microRNAs (miR-21 & miR-92a) for diagnosis of colorectal cancer patients & their relation to tumor staging as well as their use in diagnosing a benign colorectal condition and differentiating it from colorectal cancer.

## PATIENT AND METHODS

This is a prospective cohort study that included patients undergoing colonoscopic examination for lower GI symptoms at endoscopy units at South Egypt Cancer Institute and Assiut University Hospital in the period (January 2018 – December 2018). Practical work was carried out at the Clinical Pathology Department, Assiut University Hospital. The study was approved by the Institutional review board (IRB) of the Faculty of Medicine - Assiut University with IRB no. 2018-0127. All participants had informed written consent. All age groups were included. Patients with colorectal cancer that had been received chemotherapy, radiotherapy, or surgical treatment were excluded. Patients with a history of malignant tumors in other organs were also excluded from the study.

### Grouping of patients

Patients were categorized according to colonoscopic and histopathologic findings into 3 groups:

Group A: patients with pathologic-proven colorectal cancer.

Group B: patients with precancerous benign colorectal lesions.

Group C: patients with normal colonoscopic findings (Control Group).

**Diagnostic workup:** All participants were subjected to the following:

- Clinical history and clinical examination including the digital rectal examination (DRE).
- Multi-Slice CT abdomen and pelvis.
- Full Colonoscopy.
- Pathological examination of colorectal mass biopsy.

### Sample collection

Two ml of blood were sampled into EDTA coated tube centrifuged at 1000 rpm for 5 min, and then plasma was transferred into an RNase-free tube for RNA extraction. Plasma was stored at -80 °C until assay.

**Determination of plasma miR-21 and miR-92a by (qRT-PCR):** According to the manufacturer's instructions, total RNA purification (including small RNAs) was done using the miRNeasy Mini Kit (Cat. No. 217004): QIAGEN that was stored dry at room temperature (15-25°C). Real-time PCR for detection of mature miR-21 and miR-92a was done by using 7500 fast real-time PCR "Applied Biosystems"-USA. RNA extraction was carried out using miRNeasy Mini Kit (Cat. No. 217004; Qiagen - Germany), according to the manufacturer's instructions. Extracted RNA was subjected to RNA quantitation and purity assessment using NanoDrop® (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). miRNAs reverse transcription into complementary DNA was performed using miScript® II RT Kit (Cat. No. 218161; Qiagen - Germany), according to the manufacturer's instructions which were stored at -20°C.

For detection of mature miRNA, cDNA prepared in a reverse transcription reaction using miScript HiSpec Buffer serves as the template for real-time PCR analysis using miRNA-specific miScript Primer Assay (forward primer) and the miScript SYBR Green PCR Kit, which contains the miScript Universal Primer (reverse primer) and QuantiTect SYBR Green PCR Master Mix (Cat. No. 218073; Qiagen - Germany), (composed of

HotStarTaq® DNA polymerase, QuantiTect SYBR Green PCR Buffer, dNTP Mix, including dUTP, SYBR Green I, ROX™ passive reference dye and 5 mm MgCl<sub>2</sub>). Cycling conditions for real-time PCR included PCR initial activation step for 15 minutes at 95 °C, denaturation for 15 seconds at 94 °C, annealing for 30 seconds at 55 °C, and extension for 30 seconds at 70 °C. This included a total of 50 cycles.

**Interpretation of results:** Cycle threshold (Ct) values were automatically calculated using the Rotor Gene® Q software 2.1 (Qiagen - Germany). Relative gene expression levels were normalized to a housekeeping gene (SNORD 68) and the fold change (FC) of expression or relative quantitation for the targeted miRNAs was calculated using the  $2^{-\Delta\Delta CT}$  method.

**Surgical intervention:** Patients with histopathologic proven colorectal cancer or those with lesions with high-grade dysplasia underwent standard surgical resection according to the site of the mass either by conventional colectomy or complete mesocolic excision colectomy using open or laparoscopic approaches. The final staging was assigned according to results of histopathologic examination of resection specimens, reported according to AJCC TNM staging – 7<sup>th</sup> edition. Patients with familial polyposis coli (FAP) were treated by total procto-colectomy with ileal pouch-anal anastomosis. Patients with stage IV colorectal cancer were omitted from surgical resection except for those who need palliation e.g. obstructed cases.

**Statistical analysis:** Data were analyzed using SPSS- version 21. Chi-square was used to compare the difference in the distribution of frequencies among different groups. For variables with more than two categories; the ANOVA test was calculated to test the mean differences of the data that follow a normal distribution and independent sample. Kruskal-Wallis was used to compare the median difference between groups that do not follow the normal distribution. A P-value of less than 0.05 was considered significant. The P-value of post-hoc test was calculated using Bonferroni corrections. ROC curve was depicted the diagnostic performance of biomarkers for diagnosis of CRC, analyzed as the area under the

curve (AUC). Validity statistics (sensitivity, specificity, positive, and negative predictive value) were calculated.

## RESULTS

The study included fifty-two newly diagnosed colorectal cancer patients, twenty patients with precancerous benign colorectal diseases, and twenty age and sex-matched controls.

**Patients groups:** CRC group: 52 patients with colorectal cancer:

- 15 patients with CRC TNM stage I.
- 17 patients with CRC TNM stage II.
- 12 patients with CRC TNM stage III.
- 8 patients with CRC TNM stage IV.

**Precancerous group:** 20 patients with precancerous colorectal diseases:

- 7 patients with advanced adenoma.
- 6 patients with ulcerative colitis.
- 5 patients with Crohn's disease.
- 2 patients with familial adenomatous polyposis.

**Control group:** 20 individuals with normal colonoscopic findings were selected as a control group for comparison.

There was no significant difference among the 3 studied groups regarding age, gender, or occupation. However, smoking was significantly higher in CRC cases than the precancerous group and in healthy controls (Table 1).

**microRNAs levels:** Both Plasma miR-21 and Plasma miR-92a (fold change) were significantly higher in CRC group compared to both the control group and precancerous group, with a p-value < 0.001 for each. Also, they were significantly higher in the precancerous group compared to the control group, with a p-value < 0.001 (Table 2).

**Comparison of CRC biomarkers (Early CRC vs. Precancerous):** Both Plasma miR-21 and Plasma miR-92a (fold change) were significantly higher in the early CRC group (TNM stages I & II) compared to the precancerous group, with a p-value < 0.001 (Table 3).

**Comparison of CRC biomarkers (Early CRC vs. Adenoma):** Both Plasma miR-21 and Plasma miR-92a (fold change) were significantly higher in the early CRC group (TNM stages I & II)

compared to adenoma group, with p-value = 0.015 and 0.002, respectively (Table 4).

**Comparison of CRC biomarkers (Early CRC vs. Advanced CRC):** Both Plasma miR-21 and Plasma miR-92a (fold change) were significantly higher in advanced CRC (stages III & IV) compared to early CRC (TNM stages I & II), with a p-value < 0.05 (Table 5).

**Diagnostic performance of CRC biomarkers (CRC vs. Control):** To determine the optimum diagnostic cut-off value and evaluate the sensitivity of circulating plasma miR-21 and miR-92a for diagnosis of CRC vs. healthy controls, Receiver Operating Characteristic (ROC) analysis was performed. Table 6 shows the diagnostic performance data in form of sensitivity, specificity, NPV, and PPV for miR-21 (at a cutoff value of > 2.31 FC) and miR-92a (at a cutoff value of > 2.76 FC) and combined use of both markers. These results confirm that combined plasma miR-21 and miR-92a are more sensitive and have higher diagnostic accuracy and NPV than plasma miR-92a which is more sensitive and has higher diagnostic accuracy and NPV than plasma miR-21 in discriminating CRC cases from healthy controls. Both plasma miR-21 and miR-92a have the highest specificity and PPV (Table 6).

**Diagnostic performance of CRC biomarkers (CRC vs. Precancerous):** To determine the optimum diagnostic cut-off value and evaluate the sensitivity of circulating plasma miR-21 and miR-92a for diagnosis of CRC vs. precancerous benign colorectal diseases, ROC analysis was performed. Table 7 showed the diagnostic performance data in form of sensitivity, specificity, NPV, and PPV for miR-21 (At a cut-off value > 6.44 FC) and miR-92a (At a cut-off value > 5.76 FC) and combined use of both markers. These results confirm that combined plasma miR-21 and miR-92a are more sensitive, specific, and have higher diagnostic accuracy and NPV than plasma miR-92a which is more sensitive, specific, and has higher diagnostic accuracy and NPV than plasma miR-21 in discriminating CRC cases from precancerous benign colorectal cases. Plasma miR-92a has the highest PPV (Table 7).

**Correlation between CRC Biomarkers in patients' groups:**

- In precancerous cases: Both miR-21 and miR-92a revealed significant positive correlation (r-value=0.805, p-value<0.001) (Table 8).
- In CRC cases: Both miR-21 and miR-92a revealed significant positive correlation (r-value = 0.690 & p-value < 0.001). (Table 8).

**Table 1.** Socio-demographic data among the studied groups

	CR (n=52)	Precancerous (n=20)	Control (n=20)
<b>Age/years</b>			
• Mean $\pm$ SD	52.67 $\pm$ 10.7	53.15 $\pm$ 11.1	53.75 $\pm$ 13.5
• Median (Range)	51 (33 - 79)	52 (38 - 70)	53 (35 - 78)
p-value	P <sub>1</sub> = 0.720 <sup>NS</sup>	P <sub>2</sub> = 0.874 <sup>NS</sup>	P <sub>3</sub> = 0.868 <sup>NS</sup>
<b>Sex</b>			
• Male	33 (63.5%)	12 (60%)	9 (45%)
• Female	19 (36.5%)	8 (40%)	11 (55%)
p-value	P <sub>1</sub> = 0.359 <sup>NS</sup>	P <sub>2</sub> = 0.467 <sup>NS</sup>	P <sub>3</sub> = 0.384 <sup>NS</sup>
<b>Occupation</b>			
• Non-working	25 (48.1%)	10 (50%)	8 (40%)
• Working	27 (51.9%)	10 (50%)	12 (60%)
p-value	P <sub>1</sub> = 0.784 <sup>NS</sup>	P <sub>2</sub> = 0.885 <sup>NS</sup>	P <sub>3</sub> = 0.842 <sup>NS</sup>
<b>Smoking</b>			
• No	40 (76.9%)	12 (60%)	20 (100%)
• Yes	12 (23.1%)	8 (40%)	0 (0%)
p-value	P <sub>1</sub> = 0.009*	P <sub>2</sub> = 0.023*	P <sub>3</sub> = 0.017*

\*Statistically significant, <sup>NS</sup>: Non-significant

P<sub>1</sub> (Control vs. CRC), P<sub>2</sub> (Precancerous vs. CRC) & P<sub>3</sub> (Control vs. Precancerous)

**Table 2.** CRC biomarkers among the studied groups

	CRC (n=52)	Precancerous (n=20)	Control (n=20)
<b>miR-21 (fold change)</b>			
• Mean ± SD	12.91 ± 14.52	3.36 ± 2.00	1.00 ± 0.66
• Median (Range)	8.4 (0.9-84.7)	3.2 (0.5-7.8)	0.8 (0.1-2.3)
p-value	P <sub>1</sub> < 0.001*	P <sub>2</sub> < 0.001*	P <sub>3</sub> < 0.001*
<b>miR-92a (fold change)</b>			
• Mean ± SD	12.71 ± 11.05	3.08 ± 1.61	1.00 ± 0.67
• Median (Range)	9.5 (1.2-63.1)	3.2 (0.6-5.8)	0.8 (0.3-2.8)
p-value	P <sub>1</sub> < 0.001*	P <sub>2</sub> < 0.001*	P <sub>3</sub> < 0.001*

\*Statistically Significant, P<sub>1</sub> (Control vs. CRC), P<sub>2</sub> (Precancerous vs. CRC) & P<sub>3</sub> (Control vs. Precancerous)

**Table 3.** CRC biomarkers (Early CRC vs. Precancerous)

	Precancerous (n= 20)	Early CRC (n=32)	p-value
<b>miR-21 (fold change)</b>			
• Mean ± SD	3.36±2.00	9.81 ±7.97	< 0.001*
• Median (Range)	3.2 (0.5-7.8)	8.0 (0.9-32.8)	
<b>miR-92a (fold change)</b>			
• Mean ± SD	3.08 ± 1.61	9.09 ± 5.70	< 0.001*
• Median (Range)	3.2 (0.6-5.8)	7.2 (1.2-27.5)	

**Table 4.** CRC biomarkers (Early CRC vs. Adenoma)

	Adenoma (n= 7)	Early CRC (n=32)	p-value
<b>miR-21 (fold change)</b>			
• Mean ± SD	3.79±2.18	9.81±7.97	0.015*
• Median (Range)	3.9 (0.8-7.8)	8.0 (0.9-32.8)	
<b>miR-92a (fold change)</b>			
• Mean ± SD	3.45±1.66	9.09±5.70	0.002*
• Median (Range)	3.2 (0.9-5.8)	7.2 (1.2-27.5)	

\*Statistically Significant

**Table 5.** CRC biomarkers (Early CRC vs. Advanced CRC)

	Early CRC (n=32)	Advanced CRC (n=20)	p-value
<b>miR-21 (fold change)</b>			
• Mean ± SD	9.81±7.97	17.88±20.50	< 0.05*
• Median (Range)	8.0 (0.9-32.8)	11.1 (3.5-84.7)	
<b>miR-92a (fold change)</b>			
• Mean ± SD	9.09 ± 5.7	18.5 ± 14.74	< 0.05*
• Median (Range)	7.2 (1.2-27.5)	13.7 (4.4-63.1)	

\*Statistically Significant

**Table 6.** Diagnostic performance (CRC vs. Control)

	miR-21	miR-92a	Combined miR-21&92a
Cut-off value	> 2.31 FC	> 2.76 FC	
AUC	0.977	0.991	0.981
Accuracy, %	93.06	95.83	97.22
Sensitivity, %	90.38	94.23	96.15
Specificity, %	100.0	100.0	100.0
PPV, %	100.0	100.0	100.0
NPV, %	80.0	87.0	90.9

\*Sensitivity (true positives/all diseased); specificity (true negatives/all non-diseased); PPV (true positives/all test positives); NPV (true negatives/all test negatives).

**Table 7.** Diagnostic performance (CRC vs. Precancerous)

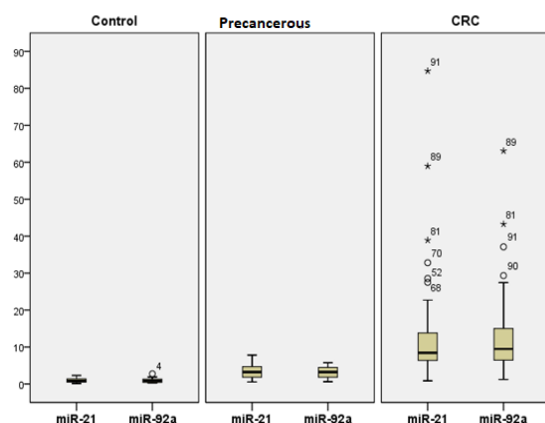
	miR-21	miR-92a	Combined miR-21&92a
Cut-off value	> 6.44 FC	> 5.76 FC	
AUC	0.867	0.927	0.917
Accuracy, %	80.56	86.11	90.28
Sensitivity, %	75.08	80.77	88.46
Specificity, %	95.0	100.0	95.0
PPV, %	97.5	100.0	97.9
NPV, %	59.4	66.7	76.0

\*Sensitivity (true positives/all diseased); specificity (true negatives/all non-diseased); PPV (true positives/all test positives); NPV (true negatives/all test negatives).

**Table 8.** Correlation between miR biomarkers

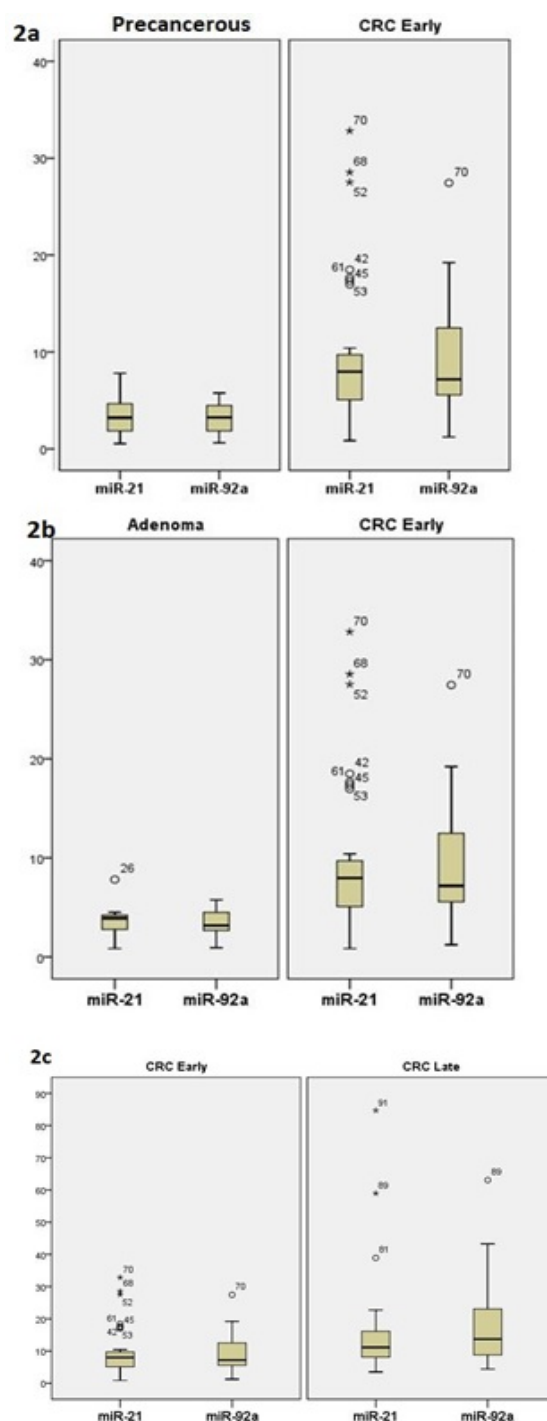
		miR-21	
Precancerous		r-value	p-value
•	miR-92a	0.805	< 0.001*
		miR-21	
CRC		r-value	p-value
•	miR-92a	0.690	< 0.001*

\*Statistically significant

**Figure 1.** Box-Plot of miR-21 & miR-92a among studied groups.

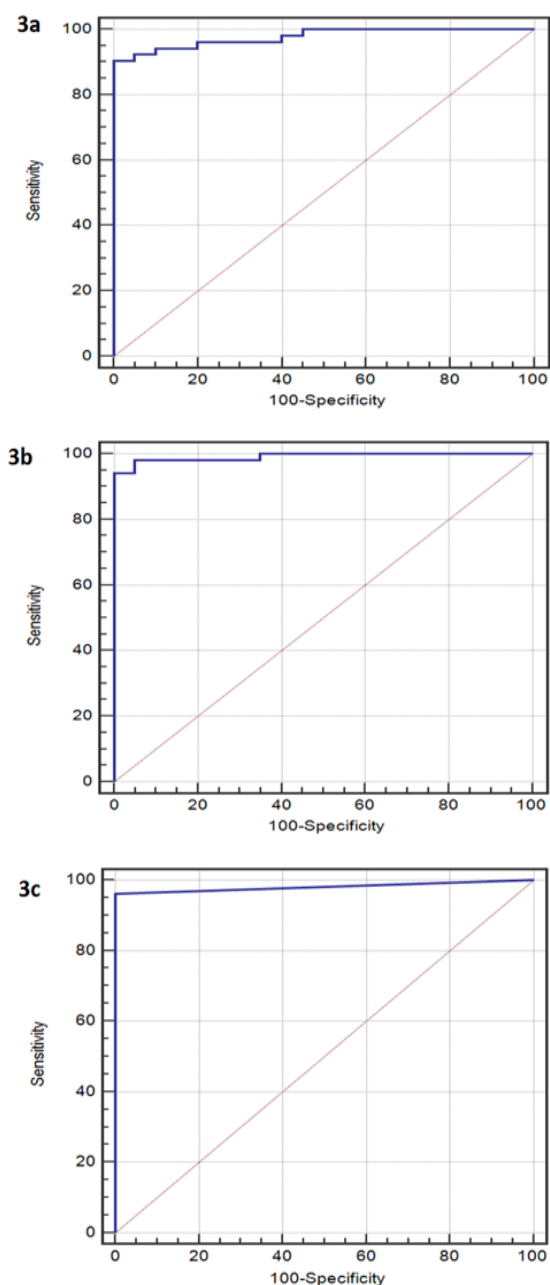
## DISCUSSION

Levels of certain microRNAs have been reported to associate with diagnosis, prognosis, metastasis, and survival in multiple malignancies (Toiyama et al. 2013; Zhang et al. 2013; Neerincx et al. 2015). Circulating microRNAs are secreted from the cells in a highly stable form that can be detected in plasma. The remarkable stability, the feasibility of detection (rapid and accurate quantification), and the direct role of microRNAs in cancer pathogenesis make them ideal non-invasive biomarkers for early cancer diagnosis (Mitchell et al. 2008). We investigated the role of measuring plasma miR-21 and plasma miR-92a for the diagnosis of colorectal cancer disease and precancerous lesions. Our results exhibited significantly higher plasma miR-21 and miR-92a in CRC patients compared to both healthy controls and patients with precancerous benign colorectal diseases, with (P-value < 0.001) for each. Also, plasma miR-21 and plasma miR-92a were significantly higher in patients with precancerous benign colorectal diseases compared to healthy controls, with P-value < 0.001 for each.

**Figure 2.** Box-Plot of miR-21 & miR-92a for Early CRC vs. Precancerous (2a), Early CRC vs. Adenoma (2b), and Early CRC vs. advanced CRC (2c).

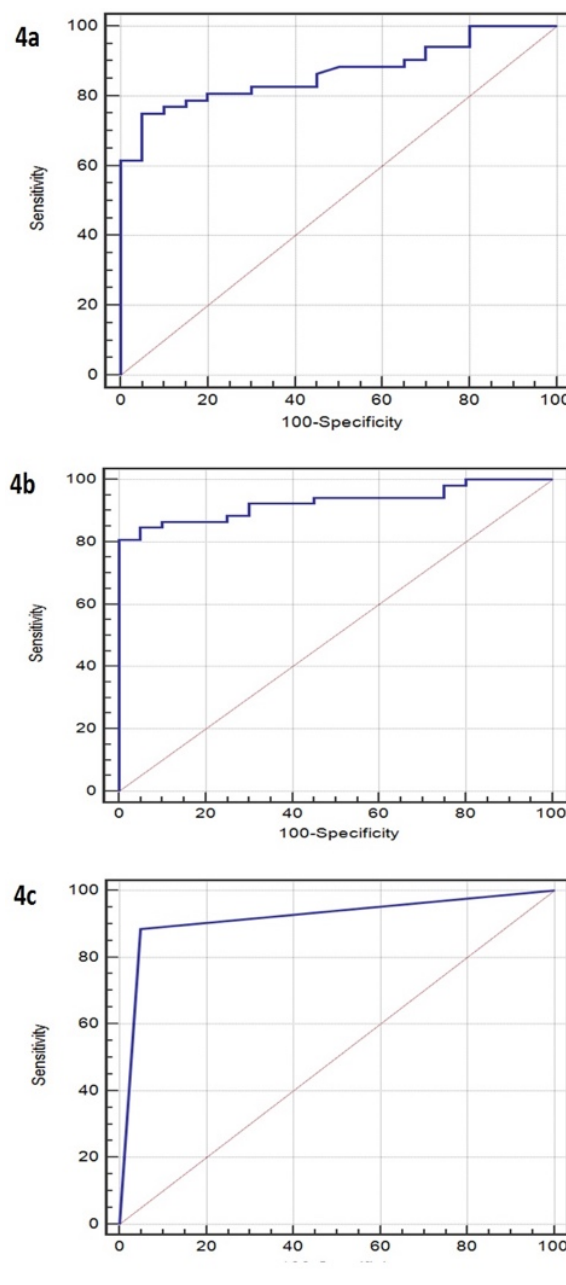
These data are consistent with those reported by many authors who found that miR-21 & miR-92a levels were significantly higher in CRC cases (Wang et al. 2014; Neerincx et al. 2015; Jepsen et al. 2016; Zhu et al. 2017).





**Figure 3.** ROC curve for CRC vs. control of miR-21 (3a), miR-92a (3b), and combined microRNAs (3c).

A recent Chinese study reported that miR-21 is significantly up-regulated in adenomas and CRC tissues, compared with the normal mucosa (Wang et al. 2016). Similarly, another study reported elevated expression of serum miR-21. This finding was validated from an independent cohort, showing that miR-21 expression was markedly upregulated in preoperative serum from CRC patients, but its expression dropped in postoperative serum from patients who underwent curative surgical resection (Toiyama et al. 2013).

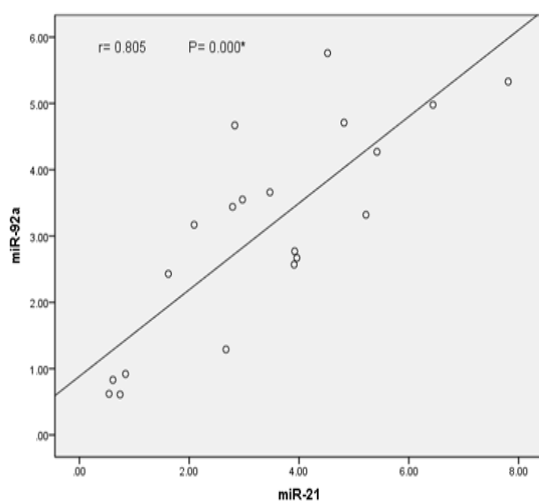


**Figure 4.** ROC curve for CRC vs. Precancerous of miR-21 (4a), miR-92a (4b) and combined microRNAs (4c).

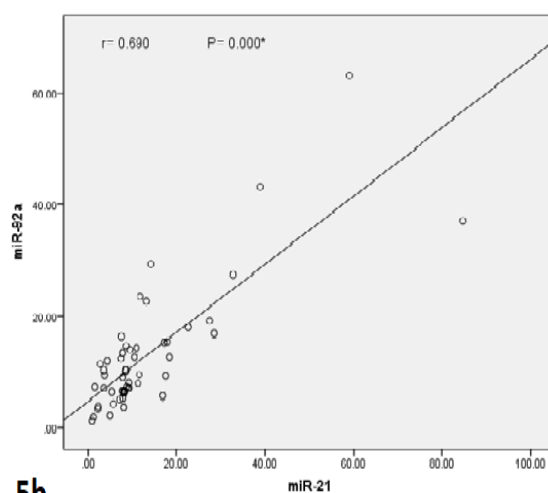
Our data showed that there was a significantly higher plasma miR-21 and miR-92a in early CRC (TNM stages I & II) patients compared to both healthy controls and patients with precancerous benign colorectal diseases, with P-value < 0.001 for each. These results were similar to Ogata-Kawata et al. who showed higher serum levels of miR-21 in primary CRC patients, even early-stage disease (TNM stage I), than in controls, and were significantly decreased after surgical resection (Ogata-Kawata et al. 2014). Recently, Zhang et al. reported that microRNA ratios; miR-21-5p/miR-

367-3p and miR-92a-3p/miR135b can differentiate CRC cases from precancerous lesions and healthy controls (Zhang et al. 2018). Another study by Lee and Ferguson reported that miR-21 and miR-92a can identify the high-risk population for colorectal cancer. Therefore, these biomarkers could be used to identify those individuals who would benefit from preventive measures as lifestyle and dietary modifications (Lee and Ferguson 2016).

In our study, plasma miR-21 and miR-92a were significantly higher in advanced CRC (TNM stages III & IV) compared to early CRC (TNM stages I & II), with P-value < 0.05 for each. This comes in agreement with Neerinx et al. who reported that the expression of miR-21 and miR-92a was associated with more advanced TNM stages (Neerinx et al. 2015).



5a



5b

**Figure 5.** Correlation between miR-21 and miR-92a in the precancerous group (5a) and CRC group (5b).

Also, this is consistent with data from other investigators who reported that higher expression levels of miR-21 were associated with more advanced clinical stages of CRC (Schetter et al. 2008, Toiyama et al. 2013). Moreover, others reported miR-92a overexpression to correlate with colorectal cancer TNM stage, lymph node and distant metastases, and decreased survival of the patients (Liu et al. 2013, Yamada et al. 2013, Zhou et al. 2013). This could aid in the surgical decision as patients with stage III colonic cancer may benefit from the adoption of complete mesocolic excision. Also, patients with stage IV colorectal cancer with no signs of intestinal obstruction are managed primarily by chemotherapy. In our study, we constructed ROC analysis for discriminating CRC cases from healthy controls. At a cut-off value > 2.31-fold change, plasma miR-21 was 90.38 % sensitive and 100 % specific, with AUC of 0.977. Also, at a cut-off value > 2.76-fold change, plasma miR-92a was 94.23 % sensitive and 100 % specific, with the area under the curve (AUC) of 0.991. Moreover, combined plasma miR-21 and miR-92a was 96.15 % sensitive and 100 % specific, with AUC of 0.981.

Our results are promising and in agreement with Said et al. who reported that serum miR-21 at a cutoff value of  $\geq 5.25$  was 94.3% sensitive and 93.3% specific for the diagnosis of CRC with an AUC of 0.995 and serum miR-92a at a cut-off value of > 6.75, was 91.4 % sensitive and 80 % specific for the diagnosis of CRC with an AUC of 0.919 (Said et al. 2017). Similarly, Guo et al. reported that serum miR-21 effectively distinguished CRCs from healthy controls with 91.6% sensitivity, 91.7% specificity, and AUC of 0.960 (Guo et al. 2018). Also, Wang et al. reported that serum miR-21 showed a sensitivity and specificity of 93% and 91%, respectively (Wang et al. 2014). Furthermore, Ahmed et al. reported plasma miR-92a to have a sensitivity and specificity of 89 % and 70 % respectively at a cut-off value > 6.75 in distinguishing CRC patients from healthy controls (Ahmed et al. 2012).

On the other hand, it was reported that the sensitivity of serum miR-21 for detection CRC was only 65% and the specificity was 85% (Liu et al. 2013). Also, Huang et al. reported similar

results with plasma miR-92a; sensitivity and specificity of 84 % and 71.4 % respectively (Huang et al. 2010). In the present work, also we constructed ROC analysis for discriminating CRC cases from precancerous benign colorectal diseases. Moreover, combined plasma miR-21 and miR-92a were 88.46 % sensitive and 95 % specific, with AUC of 0.917. These results were in agreement with Zhang et al. who reported that the microRNA ratio miR-21-5p/miR-367-3p can distinguish patients with CRC from those with adenoma, with an AUC of 0.797 (Zhang et al. 2018).

In conclusion, both plasma miR-21 and miR-92a had potential value for early detection of CRC as non-invasive screening molecular biomarkers with high sensitivity and specificity. They also help for differentiation between patients with benign and malignant colorectal lesions and those with early and advanced CRC.

#### CONFLICT OF INTEREST

Authors declare that they have no conflicts of interest.

#### FUNDING

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#### REFERENCES

- Ahmed FE, Amed NC, Vos PW, Bonnerup C, Atkins JN, Casey M, Nuovo GJ, Naziri W, Wiley JE, Allison RR 2012. Diagnostic microRNA markers to screen for sporadic human colon cancer in blood. *Cancer Genomics Proteomics* 9: 179-192.
- Aslam, M. I., Taylor, K., Pringle, J. H. and Jameson, J. S. (2009). "MicroRNAs are novel biomarkers of colorectal cancer." *Br J Surg* 96: 702-710.
- Brenner, H., Stock, C. and Hoffmeister, M. (2014). "Effect of screening sigmoidoscopy and screening colonoscopy on colorectal cancer incidence and mortality: systematic review and meta-analysis of randomised controlled trials and observational studies." *Bmj* 348: g2467.
- Calin, G. A., Dumitru, C. D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldler, H., Rattan, S., Keating, M., Rai, K., Rassenti, L., Kipps, T., Negrini, M., Bullrich, F. and Croce, C. M. (2002). "Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia." *Proc Natl Acad Sci U S A* 99: 15524-15529.
- Chen, T. H., Chang, S. W., Huang, C. C., Wang, K. L., Yeh, K. T., Liu, C. N., Lee, H., Lin, C. C. and Cheng, Y. W. (2013). "The prognostic significance of APC gene mutation and miR-21 expression in advanced-stage colorectal cancer." *Colorectal Dis* 15: 1367-1374.
- Clancy, C., Joyce, M. R. and Kerin, M. J. (2015). "The use of circulating microRNAs as diagnostic biomarkers in colorectal cancer." *Cancer Biomark* 15: 103-113.
- Croce, C. M. and Calin, G. A. (2005). "miRNAs, cancer, and stem cell division." *Cell* 122: 6-7.
- Guo, S., Zhang, J., Wang, B., Zhang, B., Wang, X., Huang, L., Liu, H. and Jia, B. (2018). "A 5-serum miRNA panel for the early detection of colorectal cancer." *Onco Targets Ther* 11: 2603-2614.
- He, L. and Hannon, G. J. (2004). "MicroRNAs: small RNAs with a big role in gene regulation." *Nat Rev Genet* 5: 522-531.
- He, L., Thomson, J. M., Hemann, M. T., Hernando-Monge, E., Mu, D., Goodson, S., Powers, S., Cordon-Cardo, C., Lowe, S. W., Hannon, G. J. and Hammond, S. M. (2005). "A microRNA polycistron as a potential human oncogene." *Nature* 435: 828-833.
- Hede, K. (2005). "Studies define role of microRNA in cancer." *J Natl Cancer Inst* 97: 1114-1115.
- Herreros-Villanueva, M., Duran-Sanchon, S., Martín, A. C., Pérez-Palacios, R., Vila-Navarro, E., Marcuello, M., Diaz-Centeno, M., Cubiella, J., Diez, M. S., Bujanda, L., Lanás, A., Jover, R., Hernández, V., Quintero, E., José Lozano, J., García-Cougil, M., Martínez-Arranz, I., Castells, A., Gironella, M. and Arroyo, R. (2019). "Plasma MicroRNA Signature Validation for Early Detection of Colorectal Cancer." *Clin Transl Gastroenterol* 10: e00003.
- Huang, Z., Huang, D., S. Ni, Peng, Z., Sheng, W. and Du, X. (2010). "Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer." *Int J Cancer* 127: 118-126.
- Jepsen, R. K., Novotny, G. W., Klarskov, L. L., Christensen, I. J., Høgdall, E. and Riis, L. B. (2016). "Investigating intra-tumor heterogeneity and expression gradients of miR-21, miR-92a and miR-200c and their potential of predicting lymph node metastases in early colorectal cancer." *Exp Mol Pathol* 101: 187-196.
- Keane, M. G. and Johnson, G. J. (2012). "Early diagnosis improves survival in colorectal cancer." *Practitioner* 256: 15-18, 12.
- Lee, K. and Ferguson, L. R. (2016). "MicroRNA biomarkers predicting risk, initiation and progression of colorectal cancer." *World J Gastroenterol* 22: 7389-7401.
- Liu, G. H., Zhou, Z. G., Chen, R., Wang, M. J., Zhou, B., Li, Y. and Sun, X. F. (2013). "Serum miR-21 and

- miR-92a as biomarkers in the diagnosis and prognosis of colorectal cancer." *Tumour Biol* 34: 2175-2181.
- Mitchell, P. S., Parkin, R. K., Kroh, E. M., Fritz, B. R., Wyman, S. K., Pogosova-Agadjanyan, E. L., Peterson, A., Noteboom, J., O'Briant, K. C., Allen, A., Lin, D. W., Urban, N., Drescher, C. W., Knudsen, B. S., Stirewalt, D. L., Gentleman, R., Vessella, R. L., Nelson, P. S., Martin, D. B. and Tewari, M. (2008). "Circulating microRNAs as stable blood-based markers for cancer detection." *Proc Natl Acad Sci U S A* 105: 10513-10518.
- Neerinx, M., Sie, D. L., van de Wiel, M. A., van Grieken, N. C., Burggraaf, J. D., Dekker, H., Eijk, P. P., Ylstra, B., Verhoef, C., Meijer, G. A., Buffart, T. E. and Verheul, H. M. (2015). "MiR expression profiles of paired primary colorectal cancer and metastases by next-generation sequencing." *Oncogenesis* 4: e170.
- Ng, E. K., W. Chong, W., Jin, H., Lam, E. K., Shin, V. Y., J. Yu, Poon, T. C., Ng, S. S. and Sung, J. J. (2009). "Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening." *Gut* 58: 1375-1381.
- Ogata-Kawata, Izumiya, H., M., Kurioka, D., Honma, Y., Yamada, Y., Furuta, K., Gunji, T., Ohta, H., Okamoto, H., Sonoda, H., Watanabe, M., Nakagama, H., Yokota, J., Kohno, T. and Tsuchiya, N. (2014). "Circulating exosomal microRNAs as biomarkers of colon cancer." *PLoS One* 9: e92921.
- Rawla, P., Sunkara, T. and Barsouk, A. (2019). "Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors." *Przegląd gastroenterologiczny* 14: 89-103.
- Said, E., Zidan, A., Nawar, A., Alhusseini, N., Soliman, E., Shaker, R., Naguib, W., Rady, K. and Baiomy, H. (2017). "Circulating Micro RNA- 21 and - 92a as Biomarkers of Colorectal Cancer." *Afro-Egyptian Journal of Infectious and Endemic Diseases* 7: 247-257.
- Schetter, A. J., Leung, S. Y., Sohn, J. J., Zanetti, K. A., Bowman, E. D., Yanaihara, N., Yuen, S. T., Chan, T. L., Kwong, D. L., Au, G. K., Liu, C. G., Calin, G. A., Croce, C. M. and Harris, C. C. (2008). "MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma." *Jama* 299: 425-436.
- Toiyama, Y., Takahashi, M., Hur, K., Nagasaka, T., Tanaka, K., Inoue, Y., Kusunoki, M., Boland, C. R. and Goel, A. (2013). "Serum miR-21 as a diagnostic and prognostic biomarker in colorectal cancer." *J Natl Cancer Inst* 105: 849-859.
- Wang, J., Huang, S. K., Zhao, M., Yang, M., Zhong, J. L., Gu, Y. Y., Peng, H., Che, Y. Q. and Huang, C. Z. (2014). "Identification of a circulating microRNA signature for colorectal cancer detection." *PLoS One* 9: e87451.
- Wang, X., Chen, L., Jin, H., Wang, S., Zhang, Y., Tang, X. and Tang, G. (2016). "Screening miRNAs for early diagnosis of colorectal cancer by small RNA deep sequencing and evaluation in a Chinese patient population." *Onco Targets Ther* 9: 1159-1166.
- Yamada, N., Nakagawa, Y., Tsujimura, N., Kumazaki, M., Noguchi, S., Mori, T., Hirata, I., Maruo, K. and Akao, Y. (2013). "Role of Intracellular and Extracellular MicroRNA-92a in Colorectal Cancer." *Transl Oncol* 6: 482-492.
- Zekri, A. R., Youssef, A. S., Lotfy, M. M., Gabr, R., Ahmed, O. S., Nassar, A., Hussein, N., Omran, D., Medhat, E., Eid, S., Hussein, M. M., Ismail, M. Y., Alenzi, F. Q. and Bahnassy, A. A. (2016). "Circulating Serum miRNAs as Diagnostic Markers for Colorectal Cancer." *PLoS One* 11: e0154130.
- Zhang, J., Raju, G. S., Chang, D. W., Lin, S. H., Chen, Z. and Wu, X. (2018). "Global and targeted circulating microRNA profiling of colorectal adenoma and colorectal cancer." *Cancer* 124: 785-796.
- Zhang, J. X., Song, W., Chen, Z. H., Wei, J. H., Liao, Y. J., Lei, J., Hu, M., Chen, G. Z., Liao, B., Lu, J., Zhao, H. W., Chen, W., He, Y. L., Wang, H. Y., Xie, D. and Luo, J. H. (2013). "Prognostic and predictive value of a microRNA signature in stage II colon cancer: a microRNA expression analysis." *Lancet Oncol* 14: 1295-1306.
- Zhou, T., Zhang, G., Liu, Z., Xia, S. and Tian, H. (2013). "Overexpression of miR-92a correlates with tumor metastasis and poor prognosis in patients with colorectal cancer." *Int J Colorectal Dis* 28: 19-24.
- Zhu, M., Huang, Z., Zhu, D., Zhou, X., Shan, X., Qi, L. W., Wu, L., Cheng, W., Zhu, J., Zhang, L., Zhang, Chen, H., Y., Zhu, W., Wang, T. and Liu, P. (2017). "A panel of microRNA signature in serum for colorectal cancer diagnosis." *Oncotarget* 8: 17081-17091.

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EACR is an NGO society that was declared by the Ministry of Social Solidarity (Egypt) No. 1938 in 19/11/2014 based on the initiative of Prof. Mohamed Labib Salem, the current Chairman of EACR. EACR aims primarily to assist researchers, in particular young researchers in the field of cancer research through workshops, seminars and conferences. Its first international annual conference entitled "Anti-Cancer Drug Discovery" was successfully organized in April 2019 (<http://acdd.tanta.edu.eg>). Additionally, EACR aims to raise the awareness of the society about the importance of scientific research in the field of cancer research in prediction, early diagnosis and treatment of cancer. EACR is also keen to outreach the scientific community with periodicals and news on cancer research including peer-reviewed scientific journals for the publication of cutting-edge research. The official scientific journal of EACR is "International Journal of Cancer and biomedical Research (IJCBR: <https://jcbjournals.ekb.eg>) was successfully issued in 2017 and has been sponsored by the Egyptian Knowledge Bank (EKB: [www.ekb.eg](http://www.ekb.eg)).

**EACR Chairman,**

**Prof. Mohamed Labib Salem, PhD**

Professor of Immunology

Faculty of Science, Tanta University, Egypt

## ABOUT JOURNAL

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International Journal of Cancer and Biomedical Research (IJCBR), a publication of the Egyptian Association for Cancer Research (EACR), is a peer-reviewed online journal published quarterly. The journal allows free access (Open Access) to its contents and permits authors to self-archive a final accepted version of the articles on any OAI-compliant institutional / subject-based repository.

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